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**THE DECOMPOSITION AND UPGRADING OF
AGRICULTURAL WASTES BY THE EARTHWORM
EISENIA FETIDA (SAVIGNY 1826).**

Submitted by David Knight to the
Open University as a thesis for the
degree of Doctor of Philosophy.
in the Faculty of Technology.

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Abstract

Cattle slurry from various sources, both digested and undigested, was tested as a culture medium for growing the earthworm *Eisenia fetida*. Earthworm growth was related to the physical-chemical characteristics of the slurry and the presence of bulk organic material.

Cattle slurry from several sites which had been mechanically separated to produce a stackable solid had different physico-chemical characteristics and produced varying earthworm growth responses. In particular, solids from Bore Place farm had a high ionic conductivity and ammonium ion concentration which reduced growth. The woodchip bedding used was found to be inhibitive to *E.fetida*.

Solids that were pretreated by high temperature composting as opposed to room-temperature aging before earthworm inoculation generally produced a higher earthworm biomass, but only after an initial inhibitory effect was overcome.

The residue of anaerobically digested cattle slurry can also be used to support *E.fetida*, although in some cases the digestion process can inhibit subsequent earthworm growth.

The addition of cellulose to cattle waste, using different cellulosic bulking agents, showed the importance of both the form of carbon present and the C/N ratio on earthworm growth, effecting both nutritional and environmental qualities of a waste. Results using an artificial cellulose based medium showed how earthworms require a complex waste ecosystem in equilibrium to survive.

Under laboratory conditions *E.fetida* does not provide a major contribution to the changes in physico-chemical characteristics during decomposition of cattle solids compared to the action of micro-organisms. The main effect of earthworms may therefore be purely physical in nature, macerating the solids and reducing particle size.

Undigested and digested worm worked material can support plant growth but requires further pre-treatment such as bulking with peat to allow plant growth equivalent to a commercial compost.

Chapter One

**The Ecology of Waste Decomposition and Upgrading to
Useful Materials; An Appraisal of the Role of *Eisenia fetida*.**

1.1) Introduction to the Thesis.

Agricultural wastes could best be described as organic materials in the wrong place at the wrong time and in the wrong amounts, and as such represent a bottleneck in the cycling of organic matter. Waste management systems are placed under great strain by the large quantities of agricultural wastes produced in the UK, putting at risk their two primary concerns, the avoidance of environmental pollution and the maximisation of the value of the wastes.

To increase the value of waste has been the subject of research for many years, and several waste upgrading systems have been proposed. Two of these are earthworm culture and anaerobic digestion, both of which treat agricultural wastes as a resource and which produce end-products of higher value than the original waste material. In the case of earthworms it is a source of protein with a high biological value, and a worm worked compost with horticultural potential. For anaerobic digestion it is biogas with a high energy value, and a slurry with improved physico-chemical qualities.

Several studies have indicated that anaerobic digestion is uneconomic at present when costing methane production and utilisation as the main output from the system (Anon 1982, James and Campbell 1983). However if the residue from the digester could be upgraded above its crude fertiliser value the economics of the system could be dramatically altered. This could be achieved if earthworms can be shown to grow and reproduce using anaerobically digested solids as the substrate, and produce viable end-products of greater value than the digester residue.

The aim of this project is to evaluate the potential of agricultural waste management using earthworms, by investigating the process of upgrading by *Eisenia fetida* and assessing one of the end-products, worm worked material. Another objective is to assess the value of anaerobic digester residue as a substrate for earthworm culture. This is because

integrating anaerobic digestion and earthworm culture can have important implications for the economic performance of anaerobic digestion.

The literature review and research concentrates on waste produced from dairy cattle before and after treatment by anaerobic digestion, and with material from different sites.

This work is part of a wider programme of research in the UK, based mainly at Rothamsted Experimental Station, but with a wide group of collaborating institutions including the Open University, involved in assessing a range of agricultural wastes as to their suitability for upgrading by earthworms, with the ultimate aim of the profitable exploitation of the process by a business.

The literature review places in context research to date on the exploitation of agricultural wastes by earthworms, followed by direct 'action research' on earthworm culture on a large scale to identify some of the problems involved. These are studied in detail in a second series of field and laboratory experiments. From this several hypotheses as to the nature of earthworm/organic waste interactions are drawn up and tested. Finally, the production of worm worked material as an end-product is investigated and evaluated as a horticultural product. With an understanding of the problems involved in waste upgrading by earthworms and an evaluation of the principal end-product of the process, the advantages and disadvantages of earthworm culture alone or in combination with anaerobic digestion as waste treatment systems can be assessed.

1.2) The Forms and Amounts of Cattle Waste Produced, and Current Disposal and Management Policies.

1.2.1) The Problem.

Organic waste can take several forms, and its presence in large quantities and concentrated form creates problems of handling and disposal. In recent years the advantages of utilising wastes as a resource have become apparent, at the same time as waste management practices have been criticised for a lack of regard for environmental effects.

The potential for earthworms to process and stabilise organic materials has been studied for several years. In Britain such research has concentrated on agricultural waste, especially animal excreta. In this section the amount of waste available, and its different forms will be discussed. Waste from cattle will be used as the example as this is the main form of waste used experimentally.

1.2.2) The Amount of Cattle Waste Produced Annually in the UK.

It is impossible to arrive at a precise figure for the total amount of cattle waste produced each year in Britain, but an estimate can be obtained from the total number of animals in the country and data for average daily excreta production.

Larkin *et al* (1981) estimated that seven million tonnes dry weight per annum of collectable waste was produced from cattle. This compares with a figure of 16.7 million tonnes dry weight quoted by Spedding *et al* (1981). The important factor in these figures is whether the waste is collectable.

Although dairy farms produce more animal waste than any other types of farm they

usually have sufficient grassland to dispose of it. However, the majority of waste is produced when animals are housed over the winter months when landspreading is impractical. If past trends towards greater intensification of livestock continue then even greater amounts of waste will be produced and require disposal.

1.2.3) Forms of Cattle Waste.

Cattle wastes are produced in three main forms, as solids, semi-liquid slurries or as liquids. Solids are normally produced when deep litter is used as bedding. Materials such as straw can absorb 1.5 to 3 times their own weight in water (ADAS 1980) and raise the solid content of the waste above 20% to produce farmyard manure, which can be stored in middens or heaps.

Recent trends in intensive agriculture have been to reduce the amount of bedding used for cattle to a minimum, to save costs. Waste produced therefore takes the form of a semi-liquid slurry with a solids content between 10-15% which must be scraped or pumped into suitable storage facilities, such as tanks or lagoons.

If slurry is diluted with dairy parlour washing water, the solids content may be reduced below 10% producing a liquid waste. At such high moisture contents handling is eased, although being more diluted greater volumes of waste will accrue.

1.2.4) Cattle Waste Management Techniques.

In a survey of dairy farm waste management policies in South-West Scotland, Brownlie and Henderson (1984) noted that virtually all manure was recycled onto the land. 95% of farmers had invested in solid manure or slurry storage facilities, and it is interesting

to report on the reasons why. The majority, 64% had slurry storage for convenience only, 19% for land protection, 10% for pollution control, and 7% to conserve crop nutrients. 30% of farmers used inorganic fertilisers without taking into account nutrients added in an organic form. On no farms was there any capital expenditure on waste treatment systems of any kind.

It is impossible to extrapolate from South-West Scotland to the rest of the UK. Certainly on larger farms in Britain (>100 cows) greater sums will be spent on storage facilities and waste handling equipment, and possibly conventional waste treatment systems.

Although receiving considerable research interest (AFRC 1983), novel waste treatment processes such as aeration, anaerobic digestion, composting and the use of earthworms have only been taken up by a tiny minority of farmers, with an even smaller proportion within the dairy industry.

Because of land availability the pressures of disposal and pollution control are not so great for dairy farmers as compared to pig and poultry farmers. The economics of any dairy cattle waste treatment system is therefore of major importance if it is to be adopted.

It would appear that most farmers still want to dispose of their waste as cheaply as possible, with the minimum investment necessary to ease waste management problems and conform to pollution regulations. Although there seems to be a growing awareness of the fertiliser potential of agricultural wastes, the initiative for a more positive approach and the treatment of waste as a resource is still lacking. With further research and development such attitudes may change.

1.3) The Physico-chemical Nature of Agricultural Animal Wastes.

1.3.1) Introduction.

Gibb and Neilsen (1976) define animal excreta as 'the undigested parts of the food intake, surplus water and the end products of body metabolism other than those yielded as milk or tissue increase. They may be excreted through the lungs, the skin or kidneys, or evacuated through the anus. Faeces consist of undigested food residues, excretory material, cell debris and bacterial residues; urine contains urea together with salts and water.' Animal waste characteristics can vary according to on the following criteria; the animal from which the excreta is derived, the sex, age, and physiological state of the animal, the diet of the animal, and environmental conditions, eg. temperature.

In addition, materials can also be added to the faeces and urine, such as bedding eg straw, woodchips, sawdust, sand etc. As well as altering the chemical properties of the waste, such bulking material can turn liquid faeces and urine into semi-liquid slurry, or solid farmyard manure. This makes animal wastes very difficult to quantify in terms of physico-chemical properties, not least in simply sampling the material in order to analyse it. However the processes of food digestion in the animal gut can be defined, and limits placed on the values of physico-chemical factors in the waste, in order to better understand the materials that may be used as the substrate for treatment.

Because the experimental work in the thesis utilises cattle waste exclusively all the examples that follow refer to this material.

1.3.2) The Digestion Process in the Animal Gut.

The cow is a ruminant, and can take advantage of microbial fermentation processes in

the rumen in order to break down high cellulose feeds. Such microbial activity has been reviewed by Hungate *et al* (1964). The faeces which leave the digestive system of the cow are made up of the following materials;

Fibre not degraded in the rumen, eg lignin and bound hemi-cellulose and cellulose.

Fibre bound and undigestible proteins.

Ammonia and ammonium ions.

Dead and living microbial cells.

Cells sloughed from the wall of the digestive system.

Water.

Various mineral salts.

Volatile fatty acids. and fibre bound lipids

Volatile sulphur compounds.

Undegraded carbohydrates.

It is worth emphasising that cattle slurry is in a constant state of chemical and physical flux, mainly through the metabolic activities of microbes, and so it is impossible to give precise figures for many of the factors within the waste, as shown by the study of the nutrients in the cattle slurry of 34 farms in Eire by Tunney and Molley (1975).

1.3.3) The Constituents of Cattle Waste: Nitrogen.

Faeces and urine contain different nitrogenous compounds, and so the relative amount of each type of excretory product in the waste is important in determining the distribution of nitrogen. Moore and Beehler (1980) studied N transformations in dairy cattle waste on storage. In the faeces alone more than 95% of nitrogen was in the organic form. However, when faeces and urine were collected, the level of total nitrogen doubled, and the levels of $\text{NH}_4^+\text{-N}$ increased more than twenty-fold. In this case just under half of the nitrogen in the faeces and urine was in the form of ammonia and ammonium ions. The addition of urine raised the pH to over 8, increasing the rate of ammonia volatilisation compared to faeces alone, which had a pH of 6.2. Although the addition of urine doubled the amount of nitrogen present in the waste, the alteration of the pH led to a 32% loss of $\text{NH}_3\text{-N}$ over a 12 week period.

Organic N, and especially urea are very quickly broken down microbially to ammonia, which is very volatile at high pH's, and can account for a significant N loss from slurries. Environmental conditions and forms of slurry storage can greatly effect the rate of N volatilisation loss (Vanderholm 1975).

In aerobic conditions the nitrifying bacteria *Nitrosomonas* and *Nitrobacter* convert ammonia (NH_3) via nitrite (NO_2^-) to nitrate (NO_3^-). (Sharma and Ahlert 1977). However, this microbial nitrification process can be inhibited by relatively high levels of free ammonia (Anthonisen *et al* 1976). In a poultry manure oxidation ditch free ammonia levels of greater than 1.0 mg/l caused an accumulation of nitrite, but no nitrate formation.

Freshly voided cattle waste will contain negligible quantities of nitrate and only some form of treatment will cause NO_3^- levels to rise. Jones *et al* (1973) analysed the forms of nitrogen in dairy waste, and produced the following results, showing the very low nitrate levels;

Treatment	NO_3^- -N and NO_2^- -N	NH_4^+ -N	Tk _j N
(all figures in mg per litre)			
Aerobic lagoon	3	251	891
Anaerobic lagoon	2	155	203
Liquid storage	5	597	1918

(Tk_jN = Total Kjeldahl Nitrogen, as determined by the macro-kjeldahl method.

See section 5.1.4)

In a study of N transformations in animal wastes Chang *et al* (1971) showed that most of the nitrogen is in the organic form on excretion, with between 40-55% N losses at 20°C over the 40 day period. Initially the organic N is converted to ammonia, with some being formed into refractory organic nitrogenous compounds, which do not undergo rapid biodegradation. Chang observes that the ammonia formed may go through a sequence of nitrification and denitrification, or it may be resynthesised microbially into organic forms. Half of all N losses are from volatilisation, and half through denitrification, with an increasing denitrification loss occurring with an increasing organic matter concentration.

1.3.4) The Constituents of Cattle Waste: Mineral Ions.

Apart from nitrogen, potassium and phosphorus are the major nutrients found in animal wastes. Secondary nutrients are sulphur, magnesium and calcium, and micronutrients include iron, manganese, boron, chloride, zinc, copper, molybdenum etc.

All these elements are to be found in varying concentrations in animal waste, depending on the diet of the animal and how the slurry was stored. There can also be variable losses of such nutrients from animal waste during storage (Vanderholm 1973). Martin

et al (1983) summarises the quantities of mineral ions in dairy manure as follows;

% of dry matter	mean value	range
Calcium	3.88	2.30-4.90
Chloride	1.72	1.19-2.20
Magnesium	0.64	0.42-1.03
Potassium	1.42	0.81-1.75
Sodium	0.42	0.32-0.53

Total organic and inorganic phosphorus levels within dairy cattle wastes are in the order of 1%, of which 97.3% is excreted in the faeces, and 2.7% in the urine (Azevado and Stout 1974).

The ratio of organic to inorganic phosphorus in cattle solids depends on the source of the material and the level of microbial activity within the waste. When in an organic form the phosphorus is bound to solids within the waste.

For potassium 70.2% is excreted in the urine (Taiganides 1983). The diet of cattle can markedly affect the concentration of potassium, as the vegetative parts of plants have higher levels of potassium than the seeds or grain. Animals fed silage based diets will therefore excrete more potassium than those animals on high concentrate diets (Cullison 1979).

1.3.5) The Constituents of Cattle Waste: Volatile Fatty Acids.

Volatile fatty acids (VFA's) are normally found in animal slurries as breakdown products. They are usually formed under anoxic conditions as the products of microbial anaerobic respiration (Allison 1978).

The majority of VFA's originate from animal faeces rather than the urine, because it is there that the precursors for their formation are found. Other soluble breakdown products include alcohols, phenols and phenolic acids (Spoelestra 1977).

The most common VFA's found in animal slurries are in order of abundance; acetic acid, propionic acid, butyric and isobutyric acid, and the branched and straight chained valeric acid. In piggery waste acetic and propionic acid have been shown to account for greater than 75% of all VFA's present, with an overall VFA concentration of 11.8g/litre (Spoelestra 1979). Formic acid has also been found in piggery slurry at very low concentrations.

Cooper and Cornforth (1978) compared the VFA content of piggery and cattle slurry. From a total of 20 samples of each, pig slurry had a mean VFA content of 5.53 g/litre compared to 3.47 g/litre for cattle slurry. Although cattle slurry had a lower VFA concentration, the order of abundance of the various VFA's is as piggery slurry.

VFA's only build up in slurries under a specific set of conditions; when the waste is anaerobic, but when methane producing bacteria within the waste are inhibited, possibly by pH, low temperature, or high levels of VFA's. If conditions are made optimal for methanogenic bacteria as in anaerobic digestion, then a high percentage of VFA's are decomposed to methane and carbon dioxide (Cooper and Cornforth 1978).

1.3.6) The Constituents of Cattle Waste; Fibre.

The digestibility of fibre by rumen micro-flora determines the faeces content of these materials. The lignin content is especially important, as it can tend to 'shield' other types of fibre from microbial breakdown through the physical relationship of the different types of fibre in the plant material.

The forage fibre analysis of Goering and van Soest (1970) can be applied to animal manures and gives a measure of the types of fibre present (see section 5.2). In reviewing the literature, Martin *et al* (1983) calculated the following mean values for fibre from dairy cattle waste;

	Mean Value
Total Soluble Nutrients	34.0%
Hemicellulose	22.3%
Cellulose	29.3%
Lignin	14.4%
Insoluble Ash	1.0%

The addition of bedding materials to the slurry can dramatically alter the fibre levels in slurries. Hills and Roberts (1981) analysed some typical bedding materials for their fibre content as shown below;

Constituent	Sawdust	Newspaper
Hemicellulose	22.9%	20.4%
Cellulose	35.8%	60.3%
Lignin	41.5%	19.6%

Although the aim of cattle housing management is to reduce the amount of bedding used to a minimum the high fibre values shown can substantially increase slurry fibre levels.

1.4) The Use of *Eisenia fetida* as Means of Treating Agricultural Wastes.

1.4.1) The Potential for Earthworms in the Waste Treatment Process.

Agricultural wastes normally represent a negative asset to the farmer who must spend money to treat and dispose of them in a safe, non-polluting manner. Although landspreading remains the most common method of waste treatment, there are now several novel waste treatment techniques available to the farmer to be used in conjunction with landspreading or to upgrade the value of the wastes.

The culture of earthworms in organic materials is one means of upgrading waste above its crude fertiliser value. If the agricultural waste in question can satisfy the environmental and nutritional requirements of earthworm species that have evolved to such habitats, they can grow and reproduce in the waste. It has been contended that earthworms work through the waste, macerating, mixing and aerating the material, which, in conjunction with the stable environment produced by the earthworm gut stimulates aerobic microbial decomposition and stabilisation of the waste. This activity is dealt with in some detail in succeeding sections of chapter one.

By feeding on raw materials and micro-organisms within the waste the earthworm biomass can 'fix' protein, and because of their size, earthworms can be mechanically separated from the solids allowing this protein to be harvested (see section 1.8).

The acceleration of microbial activity and the macerating action of the earthworms on feeding produces a worm worked material which has many favourable properties that allow it to be used as a horticultural medium (see section 1.9).

Therefore this process has the potential to produce a protein source and a horticultural growing medium from agricultural wastes, with a market value exceeding that of the

inherent fertiliser value of the material on landspreading. If the increased value of earthworm protein and worked material can produce an economic return on the capital, labour and maintenance costs incurred in producing them, then earthworm culture can be considered a viable option for the treatment of agricultural wastes.

1.4.2) *Eisenia fetida* as an Earthworm Species for Waste Treatment.

Earthworms belong to the phylum Annelida, order Oligochaeta, with ten families of terrestrial earthworms termed the Megadrili (Edwards and Lofty 1977). Earthworms have evolved to occupy a wide range of ecological niches in soils and related materials. Earthworm species with the potential to break down agricultural wastes must be able to survive in, and feed upon organic material in a fairly fresh state. Such species will be found naturally in environments rich in organic matter. Of the 37 British earthworm species or forms described by Gerard (1964) the following have habitat descriptions relating to organic matter, and could therefore be considered for waste stabilisation; *Eisenia fetida* (Savigny 1826), *Dendrobaena mammalis* (Savigny 1826), *Dendrobaena octaedra* (Savigny 1826), *Dendrobaena venita* (Rosa 1886) *f.typica*, *Dendrobaena rubida* (Sav.) *f.subrubicunda* (Eisen 1874), *Dendrobaena rubida* (Sav.), *f.tenuis* (Eisen 1874), *Lumbricus castaneus* (Savigny 1826) and *Lumbricus rubellus* (Hoffmeister 1845).

Of these species *E.fetida* is the most commonly distributed in areas of concentrated organic matter, and has evolved to exploit O.M. in soil-less situations such as compost heaps and dung. Although other earthworm species may be found in compost heaps and dung their primary ecological niche is mineral soils with high levels of organic matter, and so they do not compete well with *E.fetida*. This is confirmed by the fact that it is exceedingly difficult to keep pure cultures of other earthworm species without contamination by *E.fetida* (Edwards pers. comm.). The growth and reproduction characteristics of *E.fetida* that allow it to compete so well in organic matter are dealt with in succeeding sections.

Other earthworm species not found endemically in the British Isles have also been considered for waste utilisation, such as *Perionyx excavatus* and *Eudrilus eugenia*, which are normally found in tropical or sub-tropical habitats. Research has shown that such species may have the potential for waste stabilisation (Neuhauser *et al* 1979, Kale *et al* 1981), although they may require more carefully controlled conditions to reach their full potential.

E.fetida is therefore the most widely distributed earthworm species in organic materials, has the greatest tolerances to environmental conditions and is therefore most easily mass-cultured, as witnessed by its introduction by humans into many parts of the world (Gates 1978). Other earthworm species may have qualities that could be exploited in certain situations, and there is certainly a research need to evaluate the ecology of such species, but *E.fetida* has received the greatest scientific investigation and is generally recognised as the most suitable earthworm species for waste stabilisation at present.

1.4.3) The History of Earthworm Culture in Organic Wastes.

The culture of earthworms was initiated by the rise in popularity of fishing as a mass participation sport in Europe and North America and the subsequent demand for fish bait. Amateur fishermen continue to dig earthworm bait from the soil, but it was soon realised that the richest source of earthworms was from a compost heap, and that if such conditions could be recreated and controlled, earthworms could be grown en masse and sold. Such earthworm farms have been in existence since at least the 1940's in the United States, and can now be found world wide, normally as small businesses with limited life-spans. There is a large 'pseudo-scientific' literature to support such activities (Gaddie and Douglas 1977a, 1977b and Barrett 1975). Unfortunately earthworm growing has attracted some dubious business practices including pyramid selling.

The growing awareness of ecological concerns in the 1970's such as the threat of pollution, the energy crisis, and the need to feed an ever increasing world population stimulated the idea of using wastes as utilisable resources. This drew the attention of scientists to the potential of earthworm growth in organic wastes. Dr R.Hartenstein and colleagues at State University New York were the first to initiate a major research programme on the use of earthworms in the management of sludges at wastewater treatment plants. The many papers published by Hartenstein, Neuhauser, Kaplan *et al* are referred to throughout this thesis.

The basic ecological work carried out by Hartenstein *et al* prompted several U.S. water authorities to set up pilot plants using earthworms to stabilise sludge. Although successful in biological terms, engineering and economic considerations curtailed their operations (Collier 1978, Camp, Dresser and McKee 1981). Work continues in this area as evidenced by recent research papers (Hartenstein *et al* 1984, Hartenstein and Neuhauser 1985).

A similar research programme was set up in Britain under Dr C.Edwards at Rothamsted Experimental Station with many other U.K. institutions. The work in Britain has involved the study of earthworms in an agricultural context, to deal with the increasing problem of waste generated by agriculture as outlined in section 1.1. The work has been summarised in several reports (Edward 1983a and 1983b, Edwards *et al* 1985).

The economics of earthworm culture has also been studied (Foote and Fieldson 1982, Fieldson 1985, Fieldson 1986). Such work indicates that the worm worked wastes rather than earthworm protein represent the major source of revenue from an earthworm culture system. This has shifted the research emphasis away from optimising the earthworm biomass in wastes towards maximising the production of worm worked material with the preferred qualities for plant growth. The importance of engineering earthworm beds and other ancillary equipment has also been considered, and work at the National Institute of Agricultural Engineering has been carried out in this area. A company called British

Earthworm Technology has been set up to commercialise research work from Government institutions. Major funding has now ceased, although there is much biological and engineering research to be carried out. Whether the system is at a stage where it can be successfully exploited commercially remains to be seen.

1.5) The Ecology and Life History of *E.fetida*

1.5.1) The Distribution of *E.fetida*

E.fetida, a member of the family Lumbricidae, is widely distributed over the temperate northern hemisphere, in any substrate high in organic matter such as leaf litter, compost, decaying vegetation etc. In recent decades *E.fetida* has been the main species exploited in commercial vermiculture operations, facilitating its deliberate introduction into many countries around the world.

1.5.2) '*E.fetida*' is two distinct species.

Almost all the references in the literature refer to the earthworm species *E.fetida* or *E.foetida*. However, it has now been shown that it is in fact two distinct, reproductively isolated species which can be distinguished by their pigmentation, one with a brown and yellow striped pigmentation, and the other having a uniform brown or red pigmentation over its surface. The two species have the popular names of the 'brandling' or 'tiger' worm and the 'red' worm respectively.

Andre (1963) was the first to cross the two 'forms' as he termed them. He found the offspring had a hybrid pigmentation, and although they produced cocoons they were not viable. This indicated that there was reproductive isolation between the two forms. Bouche (1972) gave the two forms sub-specific status, naming them *Eisenia foetida foetida* and *Eisenia foetida andrei* respectively.

Roch *et al* (1980) and Vallembois (1982) showed great differences between the two species, indicating two separate genetic pools. Jaenike (1982) used an electrophoretic survey of 5 loci between the two species and found enzyme variations. He concluded that the reproductive variation between the two species was complete, despite the absence of an

externally imposed barrier to hybridisation. He termed the two species *Eisenia foetida* (Savigny 1826) and *E.andrei* (Bouche 1972).

Jaenicke argued that because of the degree of genetic difference between the two species, there may also be differences in physiology, ecology and behaviour. Such differences were observed by Sheppard (1984), who found the number of hatchlings emerging per cocoon at 24°C was on average 4.55 for *E.fetida* and 2.86 for *E.andrei*. However *E.fetida* hatchlings had a smaller birth weight than those of *E.andrei* and therefore less chance of surviving. Overall the number of progeny per cocoon and the survival of the hatchlings balanced each other for the two species, and Sheppard found no significant difference between the number of progeny surviving to maturity for the two species.

Such ecological differences lend more weight to the view that *E.fetida* or *E.foetida* and *E.andrei* are indeed two separate species. However this raises a problem, because almost without exception the literature does not refer to the two species, apart from those papers quoted above. This literature review will therefore continue to refer to the species '*E.fetida*' as one species, except in those cases when the two species show important biological differences.

1.5.3) The Life History of *E.fetida*

Earthworms have relatively simple life histories, mature earthworms laying cocoons after copulation from which emerge hatchlings which grow directly into adult specimens with no intervening stages (Edwards and Lofty 1977).

The life cycle of *E.fetida* can be defined by the following criteria; the time taken for the hatchlings to become sexually mature, the rate of cocoon production, the incubation time per cocoon, the number of viable cocoons (hatchability) and the number of hatchlings emerging per cocoon.

Such factors in the life cycle of *E.fetida* are very much under the influence of external variables such as temperature, moisture content etc, and these are dealt with in section 1.5.

Research into the life history of *E. fetida* can be summarised in the following table;

[illegible]

Time to Reach Sexual Maturity (days)

Result	Notes	Reference
511 Winter 329 Summer	Cultured in bullock droppings	Evans and Guild (1948)
49-70 49-61	Horse manure Sewage sludge	Hartenstein <i>et al</i> (1979)
<65	Sewage sludge, 18-28°C	Hartenstein (1982)
67 46	18°C 28°C	Michon (1954)

Rate of Cocoon Production (cocoon week earthworm)

3.8	Summer 13°C	Evans and Guild (1948)
5.8 2.4	4 adults in 300cm ³ sludge 16 adults in 300cm ³ sludge	Hartenstein (1979)
3	25°C	Graff (1974)
3.2 2.7	20°C 25°C	Hartenstein (1982)

Incubation Time for Cocoons (days)

91.3 36.3 27.9 23.3 22.1	10°C 17°C 20°C 20°C mean, diurnally fluctuating 25°C	Reinecke and Kriel (1981)
77	Spring	Evans and Guild (1948)
85.5 45.6 25.2 19.2	10°C 15°C 20°C 25°C	Tsukamoto and Watanabe (1977)

Number of Viable Cocoons (Hatchability) %

Result	Notes	Reference
88%	10°C	Tsukamoto and Watanabe (1977)
72.4%	15°C	
67.9%	20°C	
30.0%	25°C	
96%	20°C cocoon incubation	Reinecke and Kriel (1981)
100%	20°C mean, diurnally fluctuating	
84.5%		Graff (1974)
78.5%		Vail (1974)
27%	20°C Sewage	Hartenstein (1982)
36%	25°C sludge	
42%	30°C	
81.5%	25°C Horse manure	Neuhauser <i>et al</i> (1979)

Number of Hatchlings Emerging per Cocoon

1-4 mean max 8	Bullock droppings	Evans and Guild (1948)
1-4+ 1-3	10-15°C 20-25°C	Tsukamoto and Watanabe (1977)
1-4 mean max 8	Horse manure 25°C	
3.1 mean	20°C	Reinecke and Kriel (1981)

From the data of Neuhauser *et al* (1979), under ideal conditions an earthworm can maximally produce 5 cocoons per week, over a reproductive life of 40 weeks, thus producing 200 cocoons with 3 hatchlings emerging per cocoon, giving a total of 600 offspring over its reproductive life. It would be unrealistic to expect anything near such levels of fecundity in the field, but it gives an indication of the reproductive potential of this species.

Individuals of *E.fetida* have been kept alive in protected cultures for four and a half years (Edwards and Lofty 1977, quoting Korschett 1914). The tenuous ecological niche occupied by *E.fetida*, with its attendant risks of predation, environmental change etc makes it unlikely to survive to such an age, and in commercial culture there is no reason to keep earthworms alive for that length of time, as they stop producing cocoons, even in ideal conditions after about one year (Hartenstein *et al* 1979).

1.5.4) The Nutrition of *E.fetida* _____

Earthworms feed on organic matter from a variety of sources, microbes, dead and decaying plant remains, animal dung and well decomposed organic material in close association with mineral soil. Earthworms play an important role in the recycling of organic material, releasing nutrients for plant growth, increasing the humus content of the soil and improving soil texture.

However, earthworms are not ubiquitous feeders, and species occupying varying ecological niches will have differing diets. Pearce (1978) identified five ecological groups of earthworms, distinguished on the basis of the nature, size, and rate of consumption of mineral and organic matter from a permanent pasture in North Wales, by an examination of their gut contents. For example, *Lumbricus castaneus* and *L.rubellus* consumed material rich in undecomposed plant remains, whereas *Apporectica caliginosa* and *Allolobophora chlorotica* fed on well decomposed detritus.

E.fetida occupies a nutritional ecological niche very high in organic matter such as animal dung (Gates 1978) and its distribution in the field is directly related to the availability of organic matter such as cow pats, compost heaps, trickling filter beds and the litter layer of soils.

Given the contended commercial potential of *E.fetida* as a source of protein and to break down organic waste produced by human activities, much research effort has been expended in defining which materials can be most profitably used to produce maximum growth and fecundity from *E.fetida*, and if this species can be used to process a variety of waste materials.

Although it is known that *E.fetida* can grow and reproduce well on animal manures and sewage sludge, it is less obvious which component of the waste the earthworms are utilising.

Deposited animal excrement forms a complex microbial ecosystem, comprising bacteria, actinomycetes, fungi and protozoa (Dindal 1978). Sewage sludges, which are known to support better earthworm growth than animal manures (Neuhauser *et al* 1980) may have a very high organic component made up from living and dead bacterial, protozoal, and to a lesser extent, fungal biomass (Pike 1975). *E.fetida* is also a common oligochaete found in the trickling filter beds of sewage works (Solbe 1975), feeding on the microbial film that develops over the filter medium.

There is good circumstantial evidence that microbes form an important part of the diet of *E.fetida*, and research has been carried out to study the specific role of microbes in the earthworm diet.

Miles (1963) found that *E.fetida* would not grow normally in sterilised soil recolonised with bacteria and fungi. However, when the soil was inoculated with protozoa the

earthworms grew to 100mm in length and became sexually mature. Miles therefore inferred that protozoa were an essential part of the diet of *E.fetida*, particularly ciliate and flagellate protozoa.

Neuhauser *et al* (1980) grew *E.fetida* on cultures of bacteria, fungi and protozoa. The bacterial species *Pseudomonas fluorescens* and *Arthrobacter* species produced a weight gain over the control as did the fungal species *Aspergillus niger*, *Polyporus versicolor* and *Geophyllum trabeum*, although the gain was smaller. The greatest weight gain was observed with the protozoa *Euglena gracilis* and dead cells of *Tetrahymena pyriformis*, which would tend to support Miles's observations.

Hartenstein (1981) states that *E.fetida* will not feed on freshly voided manure until the protozoa therein have excysted and populations built up, the inference again being that protozoa are an essential part of the diet of *E.fetida*.

In vitro studies by Pearce and Phillips (1980) showed that *Colpidium campylum*, a fresh water ciliate was killed on its passage through the gut of *L.terrestris*. In studies on *L.terrestris* and *E.fetida*, Rouelle (1983) found that the amoebae *Saccamoeba stagnicola* and *Thecamoeba* species were destroyed on passing through the earthworm gut, unless protected by the presence of mineral soil. However, the amoeba *Acanthamoeba triangularis* survived ingestion by the two earthworm species by encysting. *Rhizobium japonicum*, a bacterium known to be eaten by nematodes also survived ingestion, although the author suggests that the earthworms may have been attracted to the polysaccharidal secretions of this species of bacterium.

Flack and Hartenstein (1984) cultured *E.fetida* hatchlings on 22 species of freeze dried and washed bacteria, added to a mix of cellulose and grit. No significant differences in the growth rate between gram positive and gram negative bacteria, and between human pathogenic and non-pathogenic species was discovered. Only 6 from 19 fungal species

supported growth however, and only at one-third the rate of bacteria, but three species of protozoa supported as good a weight increase as bacteria.

Interpretation of the results with respect to the diet of *E.fetida* is difficult because all the microbes were dead, washed and freeze dried and so converted to an inert mix of organic and inorganic nutrients rather than living organisms. It is impossible to extrapolate these results to the natural feeding of *E.fetida* in organic wastes.

In comparison Morgan (1984) fed *E.fetida* on pure cultures of live micro-organisms and produced completely different results. Weight increases were obtained on four species of fungi, but weight losses or death occurred when pure cultures of bacteria or protozoa were used as a food source.

Build up of toxic microbial metabolites may have been responsible for the mortalities, and this was cited by Flack and Hartenstein as the reason for washing their freeze dried microbes, but more likely *E.fetida* naturally consumes a varied microbial diet, and monocultures are either not consumed by earthworms or supply inadequate nutrients. Certainly these papers highlight a basic lack of knowledge in this area which requires much more fundamental research.

As well as feeding directly on the microbial populations found in their environment, earthworms almost certainly have symbiotic populations of microbes in their gut. The earthworm gut is a very constant environment, for example the pH may only vary from 6.3 to 7.3 along its whole length, and given this microbial growth may be expected to flourish there. This was observed by Parle (1963a) who noted a rapid increase in the numbers of bacteria and actinomycetes during the passage of food through the gut of *L.terrestris*. This is contrary to the work of Day (1950) however, who found no increase or decrease in the numbers of bacteria, actinomycetes, and fungi in the fresh castings of *L.terrestris* as compared to soil. That earthworms may selectively feed on certain fractions

of soil and plant remains makes accurate sampling of the microbial populations actually ingested by the earthworm very difficult.

Although it seems likely that earthworms possess no indigenous gut microflora (Satchell 1983), the common soil microbes can still contribute to a symbiotic relationship. Tracey (1951) noted the cellulase and chitinase activities in the gut of several species of earthworm, including *E.fetida*, and suggested that the enzymes were from both the earthworm gut wall, and microbes in the earthworm gut. Parle (1963b) showed that the gut wall of *L.terrestris* can break down cellulose to glucose in the absence of bacteria, indicating that the earthworm gut epithelial wall produces a cellulase enzyme. The evidence for earthworms producing their own chitinase enzyme was less strong in Parle's study, the author suggesting a combination of earthworm and microbial enzymes were responsible for its breakdown.

Hartenstein (1982) noted the presence of catalase, cellulase, and peroxidase enzymes in homogenised extracts of *L.terrestris* and *E.fetida*. The test organisms were not sterilised however, and so the enzyme activity may have come from the earthworms and/or the associated gut micro-flora. The high level of peroxidase activity in the earthworm gut compared to other soil macro-invertebrates led Hartenstein to assign a major role for earthworms in the humification process.

Recently Wallwork (1983) has argued that whether digestive enzymes are produced by the earthworm gut wall, or from the gut micro-flora is largely an academic question. The fact is that *E.fetida* can utilise fats, starches proteins (Laverack 1963), cellulose, chitin, and other chemicals such as oxalate and peroxides, and possibly fix nitrogen, through a combination of epithelial and gut microbial secretions, giving the earthworm the potential to break down a wide range of wastes.

1.6) Parameters Affecting Earthworm Growth in Organic Waste.

1.6.1) Introduction.

Although *E.fetida* has evolved to occupy an ecological niche with a high concentration of organic materials, there are still environmental limits outside which the earthworm species will not grow and reproduce. To use *E.fetida* as a waste decomposition organism in order to treat agricultural wastes such limits must be defined as closely as possible. The conditions in which *E.fetida* can and cannot be used to treat and upgrade agricultural wastes is of vital importance in determining its success as a waste management technique. Temperature, moisture content, ionic conductivity, pH, and ammonia and ammonium ions are discussed as the most important physico-chemical factors affecting earthworms. It is very important to stress that these factors do not operate independently of each other, and *E.fetida* is going to be influenced by a combination of these and other biological, chemical and physical factors within an agricultural waste.

1.6.2) The Effect of Temperature.

Earthworms are poikilothermic organisms, and so a change in the external temperature has a direct effect on the rate of growth, reproduction and cocoon development of earthworms by changing their metabolic rate.

The microbial metabolic heat produced by composting organic wastes inhabited by *E.fetida* in nature suggest that it may have evolved a comparatively high optimum temperature for growth and reproduction. Watanabe and Tsukamoto (1976) found a compost heap ranged from 8.5°C in February to 28°C in August, measured 30cm down. They noted that the autumn and winter populations of *E.fetida* living in field composts consisted mainly of small earthworms weighing less than 0.01g fresh weight. In spring and summer the mean weight of the population rose to a maximum before falling off again.

Watanabe and Tsukamoto inferred that the higher temperatures of spring and summer increased the growth rate of *E.fetida*, but cocoon production was adversely affected by the high and low temperatures in summer and winter.

In the laboratory Tsukamoto and Watanabe (1977) found the growth rate of *E.fetida* increased directly with temperature from 10-25°C. The hatchability of cocoons (the number of hatchlings emerging) dropped from 88% to 30%, the incubation time dropped from 85.5 to 19.2 days and the number of hatchlings emerging per cocoon decreased with decreasing temperature. The authors calculated a zero rate of cocoon development at 5.6°C.

Kaplan *et al* (1980) kept *E.fetida* in horse manure at 5 to 33°C. Maximum weight gain occurred in worms at 20 to 29°C. At 5°C, 30% of the earthworms died and the remainder only gained 25% in weight. At 33°C, 70% of earthworms died and the remainder lost weight. In comparison, *Eudrilus eugeniae*, a tropical earthworm, has been shown to have an optimum growth temperature of 29°C (Neuhauser *et al* 1979) and *Lumbricus terrestris*, a native British earthworm has a temperature optimum of 10°C (Edwards and Lofty 1977).

The weight gain of *E.fetida* grown in activated sewage sludge at 76% moisture content was highest at 28°C. At 43% moisture content, earthworms at 28°C gained no weight and the maximum weight gain occurred at 20°C. These results highlight the inter-dependence of factors such as temperature and moisture. The optimum temperature was different at varying substrate moisture contents. (See section 1.5.2).

The time taken for *E.fetida* to reach sexual maturity is temperature dependent. Michon (1954) found that *E.fetida* became mature after 67 days at 18°C and only 46 days at 28°C.

Hartenstein (1982) found that earthworms kept at 18, 20, 25 and 28°C were nearly all

sexually mature after 65 days, whereas only 15% of earthworms at 15°C and 12% at 30°C were clitellate. After 80 days nearly all earthworms at 15°C were sexually mature, but at 30°C only 36% were mature. This would indicate that low temperatures simply retard the onset of sexual maturity, whereas temperatures above 28°C inhibit the development to sexual maturity.

Hartenstein found that although sexual maturity was achieved more quickly at 25°C than 20°C, cocoon production was slightly higher at 20°C. Hartenstein's data confirmed the results of Tsukamoto and Watanabe (1977) showing cocoon development to be faster at 25°C than at 20°C. There was no significant difference in the growth rates and final weights of *E.fetida* at 18, 20, and 25°C. This would indicate that reproduction and development of hatchlings are more susceptible to temperature changes than the rate of growth.

Reinecke and Kriel (1981) carried out laboratory experiments on *E.fetida* at constant and diurnally fluctuating temperatures. 78 cocoons were produced from 10 adult *E.fetida* kept at a constant temperature of 20°C over a four week period, compared to 42 cocoons from earthworms kept at a temperature fluctuating diurnally between 12 and 28°C, with a mean of 20°C.

The mean incubation time was significantly smaller for cocoons produced and incubated at the diurnally fluctuating temperature, 23.3 days as compared to 27.9 days for earthworms produced and incubated at a constant 20°C. However at the fluctuating temperature hatchability was reduced, 2.74 as compared to 3.1 hatchlings per cocoon.

The authors conclude that to obtain the highest yield in the shortest possible time, the cocoons should be produced at a constant 20°C (giving a hatchability of 3.1 hatchlings per cocoon), and incubated at a mean of 20°C (incubation time 24.2 days) or at a constant 25°C (incubation time 22.1 days).

E.fetida cultured in the laboratory on potato waste (Edwards 1983) were found to grow faster at 25°C compared to 20°C, and produce a higher total weight. However, earthworms cultured at 30°C exhibited considerable mortality. Earthworms kept at 25°C reached maturity earlier than those at 20°C (61 days as compared to 65 days), supporting Hartenstein's figures.

1.6.3) The Effect of Moisture content on *E.fetida*.

The level of moisture in organic waste affects both its physical and chemical properties. At high moisture contents all the pores in the waste can become filled with water, reducing the rate of oxygen diffusion, and causing anaerobic conditions to arise.

It has been argued (Satchell 1981) that the need for a continuous secretion of mucus from the goblet cells of terrestrial earthworms to facilitate gaseous exchange across the epidermis makes earthworms very sensitive to external humidity levels because of the high rates of water loss from the body. However, *E.fetida* has been shown to be able to lose 60% of its body weight, equal to 70% of its body water, and still recover (Grant 1955). This is comparable to the 60% body weight loss recorded by Roots (1956) for specimens of *Lumbricus terrestris* and *Achlorotica* which survived.

Given the ability to survive quite high levels of dessication, *E.fetida* still prefers a moist environment. Satchell (1981) noted that when allowed to move freely through a column of dried, ground, rewetted cow dung exhibiting a gradient of moisture tension, 85% of earthworms were found in layers of 81-85% moisture content.

Kaplan *et al* (1980) found 70-85% moisture content produced the maximum weight gain for *E.fetida* grown in activated sludge, dependent on temperature. The greatest weight gain was achieved at 76% moisture content at 28°C although no clear relationship emerged between moisture contents and temperature from Kaplan's data.

Mortality occurred at 87 and 90% moisture content at the higher temperatures, presumably the higher temperature was increasing the metabolic activity of microbes in the waste, increasing the oxygen demand which could not be met because of pore blockage, leading to anaerobis and earthworm death. There was no mortality at lower moisture contents.

Edwards *et al* (1985) report that *E.fetida* is tolerant of moisture contents between 50 and 90%, which broadly agrees with the data of Kaplan *et al* (1980). There seems to be no literature on the effects of varying moisture content on the rate of reaching sexual maturity, and also cocoon production and development

1.6.4) The Effect of Ionic Conductivity on *E.fetida*.

Ionic conductivity gives a general measure of the concentration of all ions present in the waste. It is sensitive to temperature and the moisture content of the waste, which will directly effect the concentration of salts in the wastes.

Kaplan *et al* (1980) found that *E.fetida* would gain weight in activated sludge with a conductivity of 900 to 1500 μ S, and in an earlier study of earthworms grown in horse manure (Hartenstein *et al* 1979) conductivity levels of 1500 to 3000 μ S were recorded. However, horse manure contaminated by urine may have a conductivity of greater than 15000uS, and is toxic to earthworms.

Edwards *et al* (1985) quote 7000 μ S as the upper limit of conductivity tolerated by earthworms in organic wastes. Bryson (1984), adjusting the ionic conductivity of separated pig solids with sodium chloride (NaCl) and magnesium sulphate (MgSO₄) solutions found a mortality effect above 6500 μ S in both cases. In another study the conductivity of different wastes in which *E.fetida* was present and active, was measured. The highest mean conductivity level recorded was 6700 uS in chicken waste. Poultry wastes generally

have higher conductivity levels because of the uric acid produced by birds' excretory systems.

Ramsay (1949) showed that for *Lumbricus terrestris*, as the osmotic potential of the external medium increased, so did the osmotic pressure of the coelomic fluid. The highest external concentration used in the experiment was 1.45% NaCl, causing the coelomic fluid to have a concentration of 1.6% NaCl. These figures are equivalent to conductivities of 22000 and 24000 μ S respectively.

These results were obtained under very artificial conditions, but if they can be related to earthworms growing in organic wastes it would suggest that earthworms have the ability to regulate osmotically their internal salt concentrations even at very high external osmotic pressures. This would indicate that deaths caused by an osmotic loss of water would occur only at very high conductivities. As it is known that lower conductivities in organic wastes cause earthworm mortalities, it would seem that death through osmotic water loss only occurs in extreme circumstances, and the usual mode of death is through specific salt toxicity effects.

Certainly some salts have a specific toxicity effect at conductivity levels that are generally sub-lethal. For example ammonium ions are known to be toxic at low concentrations (see section 1.5.5) and copper salts have been shown to be lethal to *E.fetida* at low concentrations (Hartenstein *et al* 1979). The data from Ramsays paper would suggest that all deaths caused by high levels of conductivity are due to some ill-defined toxicity effect of the salts in question rather than an osmotic effect leading to a loss in body fluids.

Kaplan *et al* (1980) found that for a 0.5% addition of sodium chloride and sodium hydrogen phosphate (NaH_2PO_4) to earthworm cultures, the former caused mortality but there was merely a loss of weight in the latter. This gives a clear indication that salts

have differing toxicity levels, but Kaplans results do not show any clear trends because of a lack of replication. Differences in the solubility coefficients of salts may also account for the different earthworm responses.

Hartenstein *et al* (1981) added a range of salts in differing concentrations to fresh sewage sludge. The conductivity of salts at a 1% concentration were measured, and the effects on earthworm growth noted. The results obtained are summarised below;

Salt (1% conc)	Initial (μ S)	Final* conductivity	Effect on Earthworm Growth
NaCl	9300	10000	Significant reduction in weight over control
NaNO ₃ ⁻	7100	7400	No effect over control
KNO ₃ ⁻	5800	5700	No effect over control
CaCl ₂	3300	4300	Significant reduction
CdO	1200	2600	Significant reduction
CdSO ₄	2800	3600	Earthworm mortality
ZnSO ₄ (Hartenstein <i>et al</i> 1981)	3700	3900	Significant reduction

*After 28 days

The authors stress that it is impossible to link a particular ion to a toxicity effect, because the ions were not introduced directly to the earthworm, and may have affected the microbes in the sludge instead. From the results however, no direct link between conductivity and weight loss and death can be discerned. Some salts cause loss of weight at quite low conductivities, whereas others, such as NaNO₃ are innocuous at levels that other workers have quoted as the upper conductivity limit.

There is no doubt that ionic conductivity affects earthworm growth and reproduction, but at present the effect of conductivity on earthworms is very poorly understood.

1.6.5) The Effect of pH on *E.fetida*.

pH has long been known to affect the distribution of earthworms in the field (Satchell 1955), with different species having varying pH optima. Edwards and Lofty (1977) report that *E.fetida* is found most often in soils between pH's 7.0 to 8.0.

Experiments to find the optimum pH for *E.fetida* in organic wastes have been made

difficult by the natural buffering capacity of organic materials, so that any external alteration of the pH is typically buffered back to its original level within several hours or days.

Kaplan *et al* (1980) tried altering the pH of activated sludge with an original value of 6.4. Despite periodic acid/base additions an initial range of pH 2.0-10.0 was buffered to 5.4-8.7 over a period of 14 days. At initial pH's of less than 5 and greater than 9 all earthworms died within a week. Of the earthworms that survived, the greatest increase in weight occurred at an initial pH of 7.0.

1.6.6) The Effects of Ammonia and Ammonium ions

Ammonia is generated in large quantities from organic wastes as a breakdown product of undigested proteins from the animal gut. Tunney (1980) states that over half the nitrogen in anaerobically stored waste can be in the form of ammonia or ammonium ions. The two forms will be in dynamic equilibrium, depending on factors such as pH, but because of the ease with which ammonia in solution volatilises as a gas from wastes (Willson and Hummel 1975), it is assumed the ammonium ions will always predominate.

Ammonia levels have a profound effect on earthworm mortality. Kaplan *et al* (1980) found that concentrations of 0.1 mg/g of ammonium acetate was fatal to earthworms, compared to figures of 1.0 mg/g for other salts such as potassium and sodium acetate and potassium chloride.

Edwards *et al* (1984) states that earthworms will not enter or utilise wastes until the ammonium level falls below 0.5 mg/g. By mixing wastes with differing ammonium ion contents Bryson (1984) was able to produce a range of ammonium ion levels from 0.2 to 10.3 mg/g $\text{NH}_4^+\text{-N}$. Mortality occurred between 4.3 and 5.3 mg/g $\text{NH}_4^+\text{-N}$. It is interesting to note that the conductivity at this level was 2300 μs , well within the acceptability

limits summarised earlier (section 1.5.4).

Bryson also added NH_4Cl to acceptable pig waste to produce a range of ammonium ion levels. In this case mortality occurred between 1.0 and 2.4 mg/g of ammonium ions. The chloride ions may have made the waste more toxic at lower $\text{NH}_4^+\text{-N}$ levels, and the addition of NH_4Cl increased the conductivity to 5400 μs at 5.89 mg/g $\text{NH}_4^+\text{-N}$ which may also have contributed to increased mortality.

Bryson observed that chicken waste with an concentration of 8.69mg/g NH_3 was found to contain live earthworms, a higher level than that in amended pig solids which killed earthworms in the laboratory.

The literature would suggest that different ammonia and ammonium ion levels can cause mortality depending on the values of other factors, and indicates the great inter-dependence of variables that affect earthworms in waste. It can be argued that if an earthworm is already under stress from some factor such as moisture, conductivity etc, then a fairly low ammonium level will cause earthworm fatalities which will not occur in conditions that are acceptable to earthworms.

1.7) The Role of *E.fetida* in the Physical and Chemical Breakdown of Organic Material.

1.7.1) Introduction.

The general role played by invertebrates in the decomposition of organic wastes involves;

The mechanical breakdown of organic materials into smaller particles, so increasing the surface area for microbial breakdown.

Movement through the waste, introducing oxygen and thus increasing aerobic processes.

The stimulation of microbial activity in the waste through gut symbiotic relationships.

Browsing on microbial colonies to remove senescent organisms, and stimulate population growth.

The release of faeces and urine (high in excreted nitrogen and mineralised salts) into the waste.

(Swift *et al* 1979)

To isolate and identify the particular role played by *E.fetida* beyond the above generalities is difficult, especially as the processes initiated by earthworms and microbes are so intricately bound together.

1.7.2) The Physico-Chemical Changes Effected by *E.fetida*.

Perhaps the most complete work to date in this area is that of Hartenstein and Hartenstein (1981). They studied the physico-chemical changes effected by earthworms in activated sludge, as compared to the decomposition processes carried out by microbes alone. Their overall conclusion was that the presence of earthworms acted to accelerate and take further many of the changes that occurred to a lesser extent in equally old activated sludge.

Their results are best viewed in tabular form;

See page 44

Physico-chemical Effect

Increase in the rate of mineralization.

Increase in cation exchange capacity.

Significant decrease in total nitrogen.

Increase in C/N ratio.

Significant increase in nucleic acid and inorganic P levels.

Lower pH in earthworm worked sludge.

Reason

Increased Concentration of soil minerals as organic matter is metabolised.

A phosphatase enzyme.
(Satchell and Martin 1984)

Increase in humic acids and fulvic acids.

Increase in earthworm biomass (58-71% protein dry wt).
Losses through denitrification.

Loss of Nitrogen.

Incorporation of inorganic and amino N into nucleic acids of earthworm biomass.

Increase in CO₂ releasing carbonic acid?
Fewer ammonium and hydroxide ions?

1.7.3) Earthworm Initiated Increases in Aerobic Decomposition.

Several authors have shown very clearly that there is a significant increase in the rate of oxygen consumption in earthworm worked sewage sludge, after correction for earthworm respiration (Hartenstein and Hartenstein 1981, Mitchell *et al* 1980, Horner and Mitchell 1981). This would indicate very strongly that many of the observed physico-chemical changes can be assigned to the increased microbial activity stimulated by the macerating, feeding and tunnelling of earthworms through organic waste.

Mitchell *et al* (1980) noted higher densities of nematode worms associated with *E.fetida* in sewage sludge and linked these to the increased rate of O₂ consumption in the sludge. The authors argue that the feeding on sludge by *E.fetida* stimulates bacteriophagic nematodes which serve an important role in accelerating bacterial turnover. However, no research yet has conclusively linked an increase in microbial activity with the changes observed by Hartenstein and Hartenstein, and so the statement remains an inference.

1.7.4) Earthworm Initiated Breakdown of Organic Phosphorus.

Research by Satchell and Martin (1984) has defined more closely the link between the stimulation of microbial activity and earthworms in organic waste materials, and also shown a direct influence of microbes on the decomposition process.

They investigated the link between earthworms and the phosphatase enzyme, which converts organic phosphorus into inorganic forms, and so causes mineralization when found in soil organisms. This is especially important in organic wastes such as cow manure, which can have up to 50% of phosphorus in an organic form.

Satchell and Martin noted an increase in the rate of mineralization of organic wastes in

the presence of earthworms, most of which can be accounted for by a reduction of organic matter, as noted by Hartenstein and Hartenstein (1981). The increase in organic P was greater than could be accounted for by the above concentration effect however, and an enzyme was suspected.

Phosphatase enzyme activity was assayed in a medium of paper waste sludge with added organic phosphorus in the form of phytin. Over a pH range of 2-10, there was a peak of phosphatase activity between pH 3-5 in the absence of earthworms, and a greater peak in the presence of *E.fetida* with an additional peak of phosphatase activity at pH 9-10. The authors interpreted the peaks at pH 3-5 as microbial phosphatase activity, and those at 9-10 as the action of an alkaline earthworm phosphatase enzyme, leading to an overall increase in phosphatase activity in the presence of earthworms.

1.7.5) Earthworms and the Nitrogen Status of Organic Materials.

The most direct effect earthworms have on the nitrogen levels in organic wastes is the incorporation of substrate N into earthworm protein, leading to a net loss from the substrate.

Kaplan and Hartenstein (1977) carried out studies into the ability of earthworms to fix nitrogen, using the Postgate acetylene reduction method. Five species of earthworm were tested, including *E.fetida*, along with their natural gut microflora. In all cases the results were negative. The authors also did not detect the presence of a nitrate reductase enzyme which could reduce nitrate to nitrite, and allow internal symbionts to convert nitrite to ammonia.

Kaplan and Hartenstein conclude that increases in nitrate and ammonia in the presence of earthworms must be due to conditions other than their own metabolic activities. They

do not rule out the possibility that under certain conditions earthworms may be able to form some type of symbiotic relationship with N-fixing prokaryotes.

Although earthworms may not contribute to the increase of total nitrogen in organic wastes they certainly play a part in the conversion of organic N to mineral N. Needham (1957) showed that the nitrogenous excretion of *E.fetida* is normally in the form of ammonium ions, urea excretion only being employed in times of starvation or dehydration.

Greater levels of total N have been shown in the cast of earthworms than in surrounding soil (Lunt and Jacobson 1944, Parle 1963). However, this can be accounted for by the selected feeding of soil earthworms on materials high in nitrogen, as shown by Darwins pioneering research (1881). Nye (1955) showed that when earthworms were kept in pots and all N levels were recorded, earthworm activity did not increase the total N in the system.

Parle (1963) found that 99.6% of excreted mineral N in the casts of soil earthworms was ammonia. After 20 days the level had dropped to 60%, and Parle inferred that most of the ammonia had been nitrified to nitrate. Parle does not give a value for the soil pH in his experiments, but it could be argued that in the more alkaline conditions of animal wastes a high proportion of ammonia would be lost through volatilisation.

In conclusion it would appear that earthworms are not directly involved in any process that increases the total nitrogen of a decomposing waste, but are directly responsible for N losses through the incorporation of N into earthworm biomass, and the mineralization of organic N, principally to ammonia, and so increase the potential for N loss through volatilisation.

1.7.6) The Role of Earthworms in the Humification Process.

The development of the wide range of aromatic compounds loosely termed humus is an integral part of the decomposition process. Any evidence that earthworms or their associated gut flora are directly involved in humification is unclear, however.

Neuhauser and Hartenstein (1978) found that *E.fetida* cannot degrade lignin, but are able to convert the ring carbon atoms of phenolic compounds such as vanillin into respiratory CO_2 . The authors argue that this peroxidase catalysed reaction may enhance the polymerisation of aromatic compounds, and so increase the rate of humification.

Neuhauser *et al* (1978) found that although earthworms and their associated gut microflora cannot metabolise lignin, they can completely degrade vanillin, a breakdown product of lignin. If the correct initial lignin decomposing micro-organisms are present therefore, the presence of earthworms will have some effect on the rate of humification.

1.8) Earthworms as a Source of Protein.

1.8.1) Introduction.

The maximum commercial potential of large scale intensive earthworm culture lies in the organic plant growth medium produced from the worm processed waste, and the high biomass of earthworm which builds up in the waste material. Early commercial worm growing operations in the United States attempted to grow earthworms to supply the huge American fishing bait market valued in 1980 as \$17.5 million. However the species used, mainly *E.fetida* and *Eudrilus eugeniae*, are not suitable for bait, most fishermen preferring *Lumbricus terrestris*, collected at night in Canada by 'worm-pickers' (Tomlin 1983).

1.8.2) The Nutritional Value of Earthworm Meal.

The use of earthworms as a source of protein has attracted wide attention. Various workers have analysed the nutrient content of *E.fetida* and these have been summarised by Sabine (1983). His combined results show *E.fetida* to have a dry matter of between 13-25%, a fat content of 3-10%, and a protein content of 58-71%, all on a dry weight basis. The high levels of protein in earthworm tissue has allowed earthworm meal to be considered as a protein supplement in animal diets.

In the same paper Sabine collected several published studies of the amino acid content of earthworms. *E.fetida* protein compares well with fish and meat meal, being high in essential amino acids, especially those containing sulphur. In contrast Hilton (1983) found freeze dried *E.eugeniae* low in essential sulphur amino acids, a result disputed by Graff (1981) who found very little difference between the amino acid content of *E.fetida* and *E.eugeniae*.

1.8.3) The Theoretical Value of Earthworm Meal for Animals.

The ability of formulated earthworm meals to support animal growth can be measured in several ways. If the essential amino acid requirements of the animal are known, then the amino acid analysis of earthworm meal can be used to determine if the protein requirements of the animal are theoretically satisfied.

Such a technique was employed by Sabine (1983) to determine the relative values of worm meal and meat meal in the diets of broiler chickens, and in this case the earthworm meal was found to be a better dietary source of protein than meat meal. The more common approach is to observe the effects of including earthworm meal in animal diets.

1.8.4) Chicken Growth Trials Using Earthworms.

Earthworms have been used in a variety of animal growth trials, using pigs poultry and fish, with mixed results.

Mekada *et al* (1979) replaced soybean or fishmeal with dried *E.fetida* meal in the diet of one week old male chicks, and found no significant difference in growth with the control group. The rate of egg laying and egg quality of cross-bred hens was not significantly different from a soybean and fishmeal control group, when live earthworms were introduced into their diet. From these experiments the authors concluded that earthworms can be used practically for poultry feed as a dietary protein source.

Taboga (1980) found that the growth of chickens from one day to eight weeks fed on a maize diet with access to excess live earthworms (*E.fetida* and *L.rubellus* cultured on silage and horse manure) was not significantly different from those grown on a complete grower diet.

Harwood (1976) carried out broiler chicken trials using dried *E.fetida* cultured in broiler litter and standard broiler litter mix diets were manufactured, worm meal replacing meat meal in providing 15% of the protein content. 20 birds were grown on a worm meal based diet, and 20 on a meat meal diet. After 31 days half the birds in each group were put on a crossover diet. The trial was terminated after 51 days.

At no point during the trials were any of the weights for any of the birds significantly different, although at the end of the trial the weight for birds on the worm diet were slightly lower. The authors suggest a lower palatability in the worm meal rather than amino acid imbalances, as chickens on the worm meal diet ate consistently less than the control birds.

1.8.5) Fish Growth Trials Using Earthworms.

Less promising results have been obtained in fish feeding trials. Hilton (1983) used freeze dried *E.eugeniae* as a protein supplement in the diet of rainbow trout. The earthworm meal was found to be deficient in essential amino acids. This was corrected and the diets formulated to be isonitrogenous and isocaloric. However there was still a significant growth depression in trout fed high levels of worm meal. The author suggests that some organoleptic or chemoreceptor factor was missing in the earthworm meal to stimulate feeding in the trout, or that the balance of essential amino acids was not correct, or possibly the earthworm meal lacked some unidentified growth factor present in other fish meals.

Tacon *et al* (1983) carried out experimental feeding trials with rainbow trout using freeze-dried earthworm meal from three sources, *E.fetida*, *Apporectica longa* and *Lumbricus terrestris*. Fish grew as well or better on diets incorporating earthworm meal derived from *A.longa* and *L. terrestris*, compared to a commercially derived diet with herring

meal as the protein source. However, *E.fetida* based earthworm meal was completely unpalatable to the trout, and they exhibited no feeding response when the worm meal was presented. The authors conclude that essential amino acid imbalances seem unlikely and therefore suggest that some factor in the coelomic fluid of *E.fetida*, possibly responsible for the unpleasant fetid smell exhibited by this species when placed in a hostile environment, may be the cause of the non-feeding response. Pre-treatment of earthworms by chemicals, heat treatment and gut voiding to remove any anti-nutritional factors present may be necessary before *E.fetida* becomes palatable to trout. The well balanced amino acid content and high fatty acid levels in earthworm meal lead the authors to suggest that further research is warranted to overcome the observed feeding problems.

1.8.6) Pig Growth Trials Using Earthworms.

No earthworm culture system has yet produced enough earthworms to conduct a major pig growth trial, and the only work on the use of earthworm meal in pig rations has been carried out by Harwood and Sabine (1978) in Australia. They incorporated earthworm meal, prepared by forced air dehydration at 65°C for 4-5 hours, into the starter and grower rations of Large White pigs. After weaning, 3 pigs were fed a commercial control ration, 2 pigs were fed a trial ration incorporating worm meal, and 2 pigs a ration containing meat meal. The trial rations were made up from grain, maize oil, necessary minerals, vitamins and antibiotics, with lime and phosphate rock. Worm and meat meal were added to the rations to provide 50% of the crude protein.

With only 7 pigs in the trial, however, no statistically acceptable data was produced. The worm meal gave acceptable growth rates and produced no toxic effects, but a much larger, replicated trial is necessary to show the worth or otherwise of earthworm meal as a protein source in the diet of pigs.

1.8.7) Pre-treatment of Earthworms to Produce Earthworm Meal: Overcoming Toxicity Problems.

As noted by Stafford and Tacon (1984), the way in which earthworms are treated prior to incorporation in an animal feed is vital to its success, firstly by making the meal as palatable and nutritionally correct as possible, and secondly by avoiding any chemical and microbial toxicity problems.

The animal growth trials described earlier commonly used earthworms in two forms, live and freeze dried. For earthworms cultured in organic wastes, feeding them live presents many problems. Unless the gut content of the earthworms are voided, animals feeding on such worms will consume some of the materials used to culture the earthworm. When animal manures have been used this can have serious health implications.

Pathogens and parasites can be transferred in one of two ways by earthworms. Firstly the infecting agent can simply be in the gut of the earthworm, and so be consumed by anything eating the earthworm.

For example, Augustine and Lunt (1974) found that earthworm species, including *E.fetida*, when fed the embryonated eggs of the nematode *Ascaridia galli* passed the infection to chickens consuming them. Earthworms which were gut voided for four days or more, or treated for 16 hours with 0.5% formalin solution did not infect the chickens, indicating that the transmission of the nematode was due only to eggs and larvae passing through the digestive tract of the earthworm.

In contrast, Lund *et al* (1966) found that when single earthworms infected with *Heterakis gallinarum* were fed to young turkeys, a certain number developed the disease 'blackhead' caused by the nematode, even when they had been passed through three changes of clean soil, or gut voided for seven days on moist filter paper. Such results indicate that the

nematode larvae burrow directly into the tissues of the host earthworm, and this was found to be the case on dissection. Lund and his colleagues discovered that earthworms immersed in 1% formalin solution for 41 hours, or 1.5% nitric acid solution for 21.5 hours effectively stopped parasitic transmission.

Tromba (1955) found that *E.fetida* is a vector in the spread of kidney worm infection in swine. The disease is caused by the larvae of the nematode species *Stephanurus dentatus*, and Tromba found that when earthworms were experimentally infected with third stage *S.dentatus* larvae, the larvae were found initially in the alimentary tract, and after four days in the brown bodies of the earthworm (masses of amoebocytes).

E.fetida is therefore a facultative host in the spread of *S.dentatus* to pigs. The most effective way of preventing kidney worm infection, if earthworms were to be used as a protein source, would be to preclude the use of earthworms grown in any sort of pig manure, whether contaminated or not, as a feed source for pigs. The same applies to poultry. In this way the life cycle of such parasites can be broken.

Other toxic materials such as heavy metals, pesticide and herbicide residues, animal food additives and hormones may also become incorporated into the body of earthworms, if the material in which they are grown is so contaminated (Ireland 1983, Gish 1970 and 1973, Edwards and Lofty 1972).

Some heavy metal and organochlorine pesticides can become concentrated in the body of earthworms, making them completely unsuitable as an animal protein substitute.

By very careful screening of both the earthworms and the waste material in which they are cultured, for all the chemical and microbial toxic agents described above a healthy, safe, nutritious and palatable earthworm meal can be produced which is going to be a serious protein alternative in animal diet preparations. Although earthworm protein has

considerable biological potential, external considerations appear to preclude its utilisation at present. These include legal problems over the source of earthworm protein being an organic waste, the problems of marketing a 'worm protein', the internal economics of earthworm treatment of wastes and the politics of EEC food production. However, further discussion of these topics is beyond the scope of this thesis.

1.9) Worm Worked Material as a Plant Growth Medium.

1.9.1) Introduction

In this section, the properties of worm worked material resulting from earthworm culture are studied to assess the biological value of the material as a horticultural product. The use of waste organic materials in horticulture and agriculture is by no means a new idea. Animal manures have been used as soil organic additives for many hundreds of years until their replacement fairly recently by the use of artificial fertilisers. As the problem of organic waste disposal grows there has been an increasing trend to explore the potential of organic wastes in a horticultural context. Tester and Parr (1983) successfully utilised sewage sludge to raise several vegetable crops on otherwise marginal land, and Gray and Biddlestone (1977) utilised composted town refuse as a top dressing to poor soils for vegetable production.

Research has recently centred on using organic wastes more precisely in horticulture, usually as a partial replacement for peat in making up seeding or potting composts. In the United States much research has been carried out with composted sewage sludge and ornamental crops, which avoid the problems of heavy metal accumulation or pathogen contamination, which can effect edible crops.

Wootton *et al* (1981) grew marigold, zinnia, and petunia in composted, digested sewage sludge. They found no nutrient deficiency or toxicity symptoms, although shoot growth was increased by NPK fertiliser additions. Chaney *et al* (1980) grew marigolds in admixtures containing sphagnum peat moss, vermiculite and different proportions of digested composted sewage sludge. The sludge was found to be an effective ingredient in potting mixes when N fertiliser was added.

The growth of vegetable crops requires constant attention to heavy metal and pathogen problems. Cheung and Wong (1983) used activated sludge, digested sludge, chicken manure, and pig manure as a soil additive for the growth of chinese cabbage. Better growth was observed in animal manures than in activated sludge, and crops grown in the latter showed heavy metal accumulation and some anatomical abnormalities.

The use of worm worked material in horticulture must be assessed according to the following criteria if it is to become a viable commodity in the field of horticultural composts;

The material must be free from human health hazards, most usually in the form of viral, bacterial and protozoal pathogens, and any heavy metal toxicity problems.

The material must be free from plant pathogens, especially nematodes, and also weed seeds.

The material must support adequate growth of the particular species of plant for which it is to be marketed, with any additives the final product may contain. Plant nutrients and micro-nutrients must therefore be in the correct form and balance.

The material must satisfy the subjective criterion of what horticulturalists and gardeners consider a 'good compost', with respect to smell, texture, particle size, colour etc.

The manufacturer of the compost must be able to guarantee a consistent, uniform product, from season to season and from year to year.

A high moisture holding capacity.

Good aggregate water stability.

No heavy metals, pesticide or hormonal residues within the compost.

Although horticultural composts based on worm worked material are now to be found on the UK market, the lack of published research suggests that many of the criteria listed above require further work before worm worked materials establish a permanent niche in the horticultural market.

1.9.2) Earthworm Catalysed Changes in Organic Materials Which Improve its Qualities as a Horticultural Medium.

In general earthworms directly, or more usually indirectly through microbial stimulation, transform organic materials so that they are more likely to satisfy the above criteria than the fresh material.

It is not intended to discuss the various merits and demerits of conventional composting compared to worm working in this section, save to mention that such a debate exists, but rather review earthworm catalysed changes in wastes which are relevant to its final use as a horticultural product.

Malodour.

The bad smells associated with organic materials are almost always the intermediate metabolic products of anaerobic microbial populations in sub-optimal conditions, such as ammonia, volatile fatty acids, volatile amines etc (Spoelestra 1980). Earthworms have been shown by several workers (Mitchell *et al* 1980, Hartenstein and Hartenstein 1981, Horner and Mitchell 1981) to increase the rate of aerobic processes within organic wastes, and so break down odorous materials and inhibit their further formation.

Aerobic processes stimulated by the presence of earthworms also break down volatile sulphur species such as H_2S , which can add to the malodour of organic materials (Waugh and Mitchell 1981). Furthermore, earthworms have been shown to accelerate the rate of humification of organic materials (Neuhauser *et al* 1978), and it is the accumulation of such humic acids which give good composts their 'earthy' smell, an ephemeral quality, but none-the-less important in marketing a commercial compost.

Plant Nutrients.

The effect of earthworms on plant nutrients in the soil has been a subject of research for many years. It is generally accepted that the casts of earthworms are higher in available nutrients than the surrounding soil. Lunt and Jacobson (1944) found higher levels of nitrate nitrogen, available potassium and phosphorus, and exchangeable calcium and magnesium in the casts of earthworms compared with the surrounding ploughed field soil. Similar results were obtained by Graff (1971). By specifically feeding on materials with a high organic matter content within the soil earthworms tend to concentrate the associated organic matter and nutrients in their casts.

With earthworms cultured in organic wastes such a feeding pattern effect cannot occur as the substrate is uniformly rich in nutrients. There is not such a clear difference between the nutrients found in the original material and the resultant worm worked material.

Several workers have observed a general increase in the rate of mineralization of organic materials on worm working (Hartenstein and Hartenstein 1981, Satchell and Martin 1984), attributed to an increase in the rate of organic matter decomposition.

The fate of nitrogen and phosphate during mineralization has been studied in detail. There is an overall loss of nitrogen on worm working (Hartenstein and Hartenstein 1981), some N being incorporated into earthworm biomass and some N being volatilised as ammonia, the main N excretory product of *E.fetida* (Needham 1957).

There is however indirect evidence to indicate that earthworms stimulate the nitrification of NH_3 to NO_3^- and so conserve N in a plant available form, possibly by browsing on senescent microbial populations, maintaining aerobic conditions, or providing a stable environment for *Nitrosomonas* and *Nitrobacter* species in the earthworm gut or casts.

However no research has been published which conclusively shows higher NO_3^- levels in

the presence of earthworms compared to microbial decomposition only. Earthworms have been shown to increase the rate of mineralization of organic phosphate through the action of phosphatase enzymes (Satchell and Martin 1984), increasing the amount of plant available phosphate in worm worked material.

Waugh and Mitchell (1981) found an increase in carbon-bonded sulphur when sewage sludge was decomposed by earthworms, and the elimination of plant toxic sulphides and volatile sulphur products. The authors suggest that carbon-bonded sulphur is readily degradable by microbes to release plant available sulphates.

There is little data on the effects of earthworm decomposition on the levels of potassium, calcium, magnesium and micronutrients in the resultant worm worked materials.

1.9.3) Pathogenicity of Worm Worked Material.

The role played by *E.fetida* in minimising the transmission of pathogens from waste to plant growth medium remains unclear. Ellis and McCalla (1976) describe how aerobic processes destroy most pathogens and parasites within days if they cannot reinfect another host. In the competition for resources by a large number of microbes, stimulated by the aerobic conditions created by earthworms, pathogens and parasites are ill-adapted and soon die.

Brown and Mitchell (1981) studied the survival of the bacterium *Salmonella enteritidis* in the presence of *E.fetida*. In laboratory cultures the decrease in *Salmonella* was 29% per day when *E.fetida* was present, compared to 14% per day in their absence.

Day (1950) found that the bacterial species *Serratia marcescens* was completely destroyed when it passed through the digestive tract of *Lumbricus terrestris*. *S.marcescens* is

a common soil and water inhabitant, but is physiologically similar to other gut inhabiting enterobacteriaceae, such as *Escherichia coli*, *Salmonella*, and *Shigella* (Stanier *et al* 1971).

Such work indicates that the decomposition of organic waste by earthworms accelerates normal enteric microbial destruction through competition from aerobic microbes, but whether this accelerated pathogen destruction removes all health hazards for anyone handling earthworm derived horticultural products must remain as speculation until further research is carried out, possibly to define minimum health standards such products should conform to. The partial sterilisation of worm worked composts by heating to 60-80°C is recommended to kill residual earthworms and cocoons and lessen pathogen problems. Golueke (1982) found such temperatures were effective in killing pathogens during normal aerobic composting.

1.9.4) Plant Growth Trials in Worm Worked Material.

Very few plant growth trials have been carried out and reported in the literature using worm worked material. Scott (1986) has published results of the use of worm worked material as a supplement to peat in loamless composts for hardy nursery stock, using cattle and pig based materials.

The container grown nursery stock species, usually conifers, responded better to cattle based worm composts than to pig. Overall, 25-50% worm worked cattle waste mixes produced as good or better dry matter increases as the peat/sand controls. Pig based composts had a higher ionic conductivity than cattle material, although the author was not convinced this was the only cause of poor growth. Earlier analyses indicated ten times more copper in pig compared to cattle waste, this may have reached toxic levels in the compost.

The main difficulties encountered by Scott seem to be generally similar to other users of organic waste, namely the inherent variability of the product, leading to problems in calculating the level of fertiliser addition required. High levels of phosphate especially gave rise for concern. An analysis of each batch would be required to calculate fertiliser needs, which for hardy nursery stock are given as single additions of slow release fertilisers.

The author concludes that product variability is the major drawback to widespread commercial use of worm worked materials at present.

Edwards *et al* (1985) published plant nutrient analyses of six worm processed animal wastes. When compared to the Fisons Levington compost control, all the worm worked wastes were found to be higher in nutrients, especially the poultry wastes. Cattle solids have the most balanced nutrient composition, whilst others have high P levels, confirming Scotts work.

Edwards *et al* describe the successful growth of a wide number of vegetables, bedding plants and ornamental shrubs in undiluted wastes or in mixes including peat, pine bark or loam, and also suggests several potential markets, including organic growers, as a tree or shrub planting medium, as well as a general purpose plant medium.

Such research shows the potential of worm worked wastes as compost substitutes in horticulture, but a great deal further work is required.

1.10) Summary of the Literature Review.

It has been shown that animal wastes in the UK create major problems of disposal, and cattle slurry is a significant part of this. However, with a knowledge of the constituents of cattle slurry its potential as a utilisable resource can be exploited.

The upgarding of cattle wastes by earthworms represents one means of maximising its usefulness to produce a source of protein and a horticultural medium, although in practice economic considerations suggest that the worm worked cattle material is the most likely viable end product

The literature concerning the life history and ecology of *E.fetida* is reviewed to show it to be currently the best species for use in such a system, and to better define its abilities to enhance the decomposition and upgrading of cattle wastes.

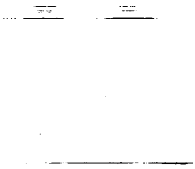
The biological value of earthworm protein is discussed and potential pathogen problems outlined. The limited data on worm worked material as a plant growth medium is assessed, with the conclusion that it has the potential to compete as a horticultural peat-like compost, but that the main disadvantage is the variable nutrient content of the material.

The literature review has shown that there is no data available on the combination of earthworm culture with anaerobic digestion, to produce an integrated system for waste treatment, nor any data on large scale management of earthworm populations suggesting a fundamental appraisal of such a system.

The overall conclusion is that although earthworms have beneficial effects upon the quality of agricultural wastes there are several problems involved, both in the process of earthworm mediated decompositon of cattle solids and in the utilisation of worm worked material as a horticultural product that requires further detailed research.

Chapter Two

Initial Experiments in Field Scale Earthworm Culture.



2.1) Introduction.

Waste management utilising earthworms depends on the correct techniques for intensively culturing earthworms to produce the desired end-products. There are several aims and objectives such a system may have;

To maximise the amounts of agricultural waste that such a system can utilise

To increase the conversion efficiency of earthworm protein from organic materials.

To increase the reproductive rate of earthworm populations for maximum gain in numbers.

To achieve high earthworm biomass per unit area of earthworm bed.

To produce a short turnover time in converting fresh organic waste into an earthworm worked material.

The production of a earthworm worked material with certain desirable qualities, including the correct balance of nutrients, particle size, moisture holding capacity etc.

There are also important economic, commercial and marketing considerations to be taken into account, but they are beyond the scope of this thesis.

In chapter one the literature concerning the life cycle, ecology, growth and fecundity of *E. fetida* was reviewed, but generally the results referred to experiments carried out in the laboratory. With the more complex interactions occurring in the field the results obtained for *E. fetida* in the laboratory should not be directly extrapolated to the field situation.

Field scale experiments in this chapter are carried out on a working farm which was actively involved in the concept of integrating the biological systems occurring on the farm (Crocker 1985). The aim of the experiments is to gain an understanding of the problems of earthworm bed management and to indicate areas of earthworm/organic waste ecology requiring further examination, and to gain 'hands-on' experience of earthworm culture. Such experiments also include an initial comparison between the ability of

digested and undigested cattle solids to support earthworm growth.

2.1.1) Description of field site.

Bore Place covers 156 ha of Weald clay in North Kent, providing grazing for 350 Friesians in summer plus 2800 tonnes of silage for winter feeding. The herd is wintered under cover for 200 days of the year and during this period receives a complete diet based on silage with added concentrates and by-products when available. The 300 housed cattle produce 13 000kg of slurry daily, which is scraped 5 times per day into a 300m³ anaerobic digester, and the digested residue then stored in a 64 000m³ lagoon. In spring the lagoon is stirred and its contents analysed for nutrient value. The digested slurry is then spread during the growing period of the grass. The farm can ~~be seen~~ on photograph 2.1.1.1.

2.1.2) Description of Below Ground Digester.

The digester at Bore Place was installed in 1979. The original capacity was 220m³, but this was increased to 300m³ to give a greater retention time for the slurry. For more details consult Dodson (1981).

2.1.3) Description of Bore Place Slurry Separator.

All experiments at Bore Place utilise mechanically separated cattle crumb derived from fresh and anaerobically digested cattle slurry. The theory behind slurry separation is described by Pain *et al* (1978), Hephherd (1980) and Gilbertson and Niebaner (1978). The separator in use at Bore Place is of a continuous belt roller design, as seen in photograph 2.1.3.1.

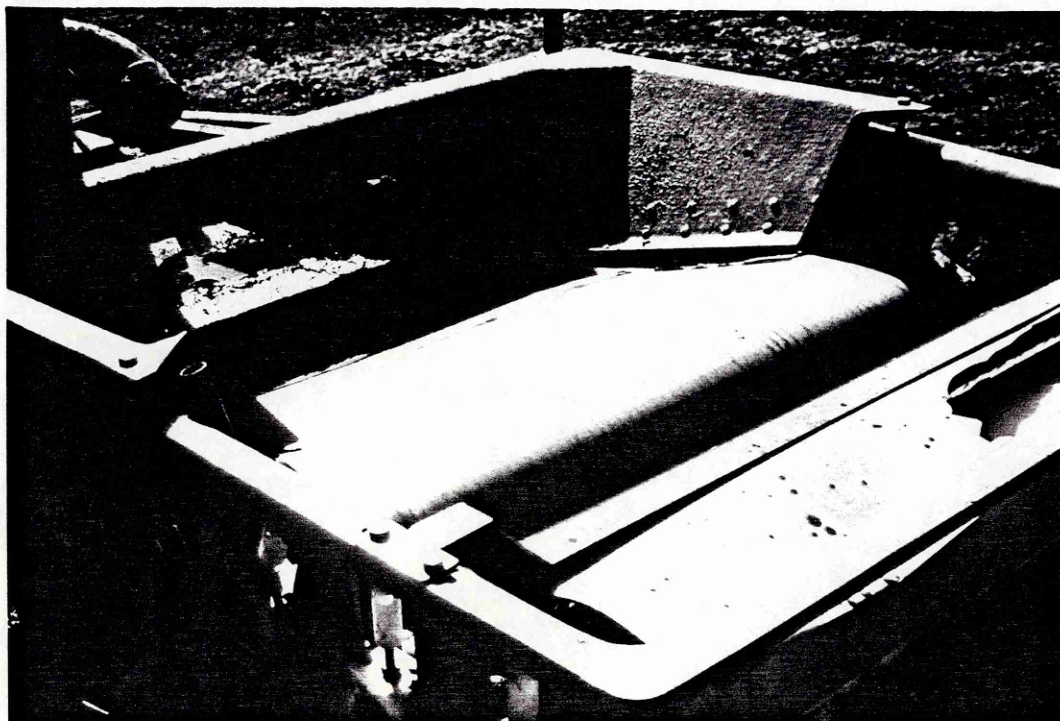
Photograph 2.1.1.1.

Bore Place Farm Showing the Cattle Sheds,
Anaerobic Digester, Gantry for the Slurry Separator,
Slurry Lagoon and Earthworm Tunnel.



Photograph 2.1.3.1.

The Continuous Belt Slurry Separator in Use at Bore Place.



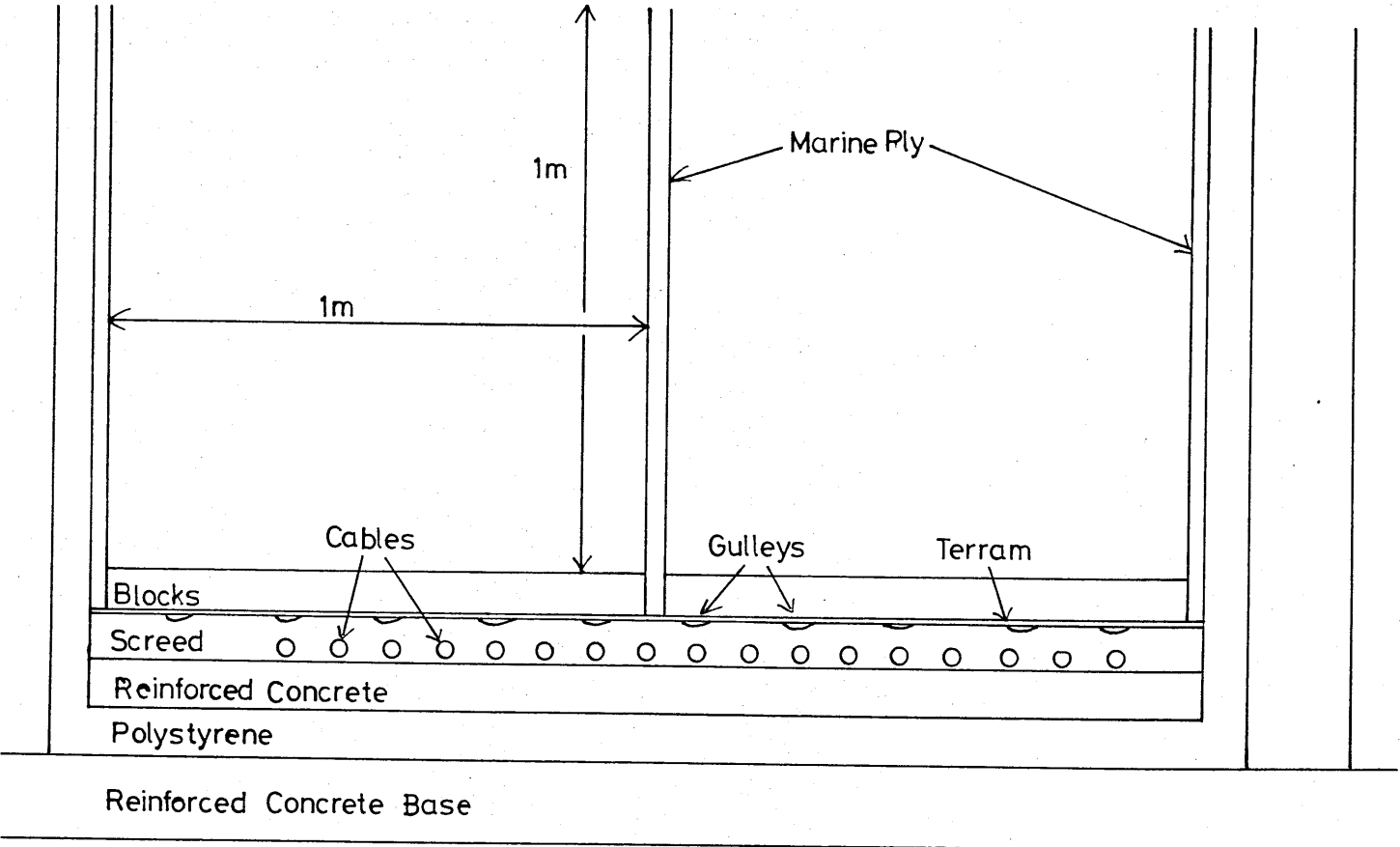
2.1.4) Description of Bore Place Earthworm Tunnel.

The experimental earthworm beds are situated adjacent to the anaerobic digester in a 6 by 15m black polyethylene tunnel with 20cm of insulating fibre between its double skin. The earthworm bed has an outer wall constructed of breeze blocks, 2m by 10m by 1.5m high. The base of the earthworm beds are made from concrete with channels etched into its surface to provide drainage with the beds built at 2 degrees from horizontal to facilitate the removal of water. Embedded in the concrete are two independent heating cables running up each side of the bed. Above the concrete is a layer of ICI 'Terram' fabric to allow the passage of water but not earthworms, and also a layer of breeze blocks to provide thermal mass for the system.

The 2 by 10m area of worm bed is split into 20 1m² beds by means of a wooden boarding 1m high. Each bed is lined with 'Terram' to inhibit the movement of earthworms from one bed to another.

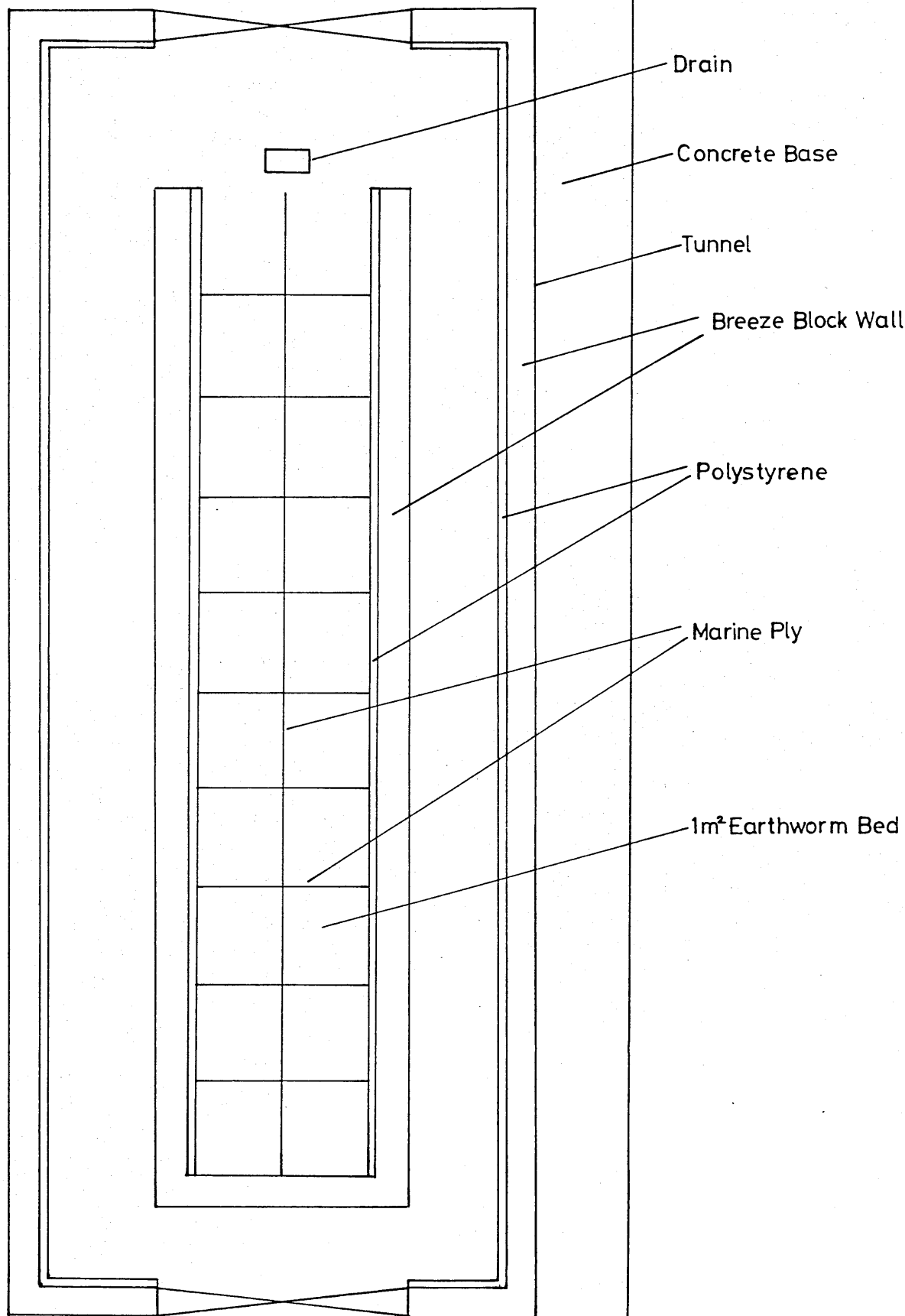
The layout of the bed can be seen in figures 2.1.4.1 and 2.

Figure 2.14.1. Plan of Earthworm Tunnel
at Bore Place



CROSS SECTION

Figure 2.14.2. Layout of Earthworm Beds
at Bore Place



2.2) The Effect Of Varying the Solid Top-loading Rate On Field Earthworm Populations

2.2.1) Introduction.

The aim of this experiment is threefold; to determine the effect of solid toploading rate on actively working earthworm beds on population gain, to obtain basic data on earthworm population dynamics in a field situation, and to compare growth rates in separated solids from digested and undigested solids. The hypothesis in this experiment is that unless other factors become limiting, increasing the amount of substrate available should increase the population of *E.fetida* present.

After an initial 25kg of digested or undigested solids in each bed ~~was~~ ^{was} ~~inoculated~~ ^{inoculated} with earthworms the experimental treatments each received subsequent toploads of the appropriate solid at the rates shown in the following table;

Undigested and Digested Solid Toploading Rates.

Treatment	Relative Rate	Actual Rate Undigested Solids	Actual Rate Digested Solids
1	x	0.025m ³ every 4 weeks	0.0375m ³ every 4 weeks
2	2x	0.025m ³ every 2 weeks	0.0375m ³ every 2 weeks
3	4x	0.025m ³ every week	0.0375m ³ every week
4	8x	0.05m ³ every week	0.075m ³ every week
5	12x	0.075m ³ every week	0.1125m ³ every week

2.2.2) Pretreatment of Solids Prior to Inoculation.

Approximately 8m^3 of undigested and digested solids was collected by passing the appropriate cattle slurry through the mechanical separator as described in section 2.1.4. The freshly separated cattle solids were found to be completely toxic to earthworms and required pre-treatment prior to the solids becoming acceptable to earthworms.

The experiment was carried out in the winter and so the two different types of solid were stored within the insulated earthworm tunnel, along each side of the experimental beds, to avoid the low ambient temperatures which may be encountered in the open, and possibly inhibit microbial decomposition processes.

The slurry separator settings were subsequently found to be incorrect, producing undigested solids with a very high moisture content (see table 2.2.2.1).

Table 2.2.2.1 Analysis of Separated Solids.

	Total Solids (%)	pH	Bulk Density (Kg/m^3)
Digested solids	27.6	7.8	526
Undigested solids (initial separation)	20.3		
Undigested solids (2nd separation)	21.8	8.5	747

(Samples oven dried overnight at 105°C)

There was a noticable difference in the structure and texture of the two types of separated solid. The digested solids had a light, open structure with well defined pore spaces within the the crumb. Undigested solids displayed less structure, with a poorly defined particulate arrangement and fewer pores. The difference in the pore size of digested solids can be measured by the lower bulk density of the material. With a higher moisture content

this made the material more prone to waterlogging and thus anaerobiosis. A second batch of undigested solids was therefore collected with a slightly lower total solids value of 21.8%. This decrease was enough to improve the structure of the solid and allow microbial decomposition to take place, suggesting a threshold level of moisture content exists for the maintenance of aerobic conditions in the separated solids.

These results indicate that the different structure of digested and undigested solids is not a consequence of the mechanical separator settings but is fundamentally related to the physical particle size of the two slurries. Anaerobic digestion leads to a breakdown of material within the slurry and a consequent reduction in total and volatile solids (Pain *et al* 1984). However lignified material is not decomposed on digestion (Robbins *et al* 1979), and so the woodchips used in the bedding at Bore Place would pass through an anaerobic digester unchanged, whereas material of a smaller particle size is broken down. Digested slurry therefore has a higher proportion of total solids with a larger particle size compared to fresh slurry, possibly leading to the observed physical differences.

2.2.3) Inoculation of Earthworm Beds.

After separation and storage along the sides of the earthworm tunnel the solids were regularly tested with mature earthworms for survival, but both types of solid were found to be very toxic. High ammonia concentrations were judged to be a possible cause of unacceptability and so action was taken to accelerate ammonia losses from the solids by volatilisation.

Within the enclosed space of the insulated polytunnel ammonia could be detected by smell, and so holes were drilled into the doors of the tunnel to improve ventilation, and the solids turned three times per week to increase the rate of ammonia volatilisation.

After 11 weeks the material had become acceptable to earthworms and so inoculation could commence. The beds were each filled with a thin layer of solids into which the initial earthworm inoculum could be placed. To take into account differences in density, a 2.5cm depth of undigested solids and 3.75cm depth of digested solids was used.

8kg of *E.fetida* were purchased from British Groundbaits of Braintree, Essex, packed in peat and with an average of 2480 individuals per kg. This total was divided into 17 sub-portions containing 447g of earthworms. The mean earthworm weight was calculated at 0.4g, giving approximately 1120 individuals per inoculum.

The initial inocula of earthworms and peat were spread evenly over the surface of the solids in each bed. No solids were added to the beds for a period because of the demands of another experiment that was later abandoned.

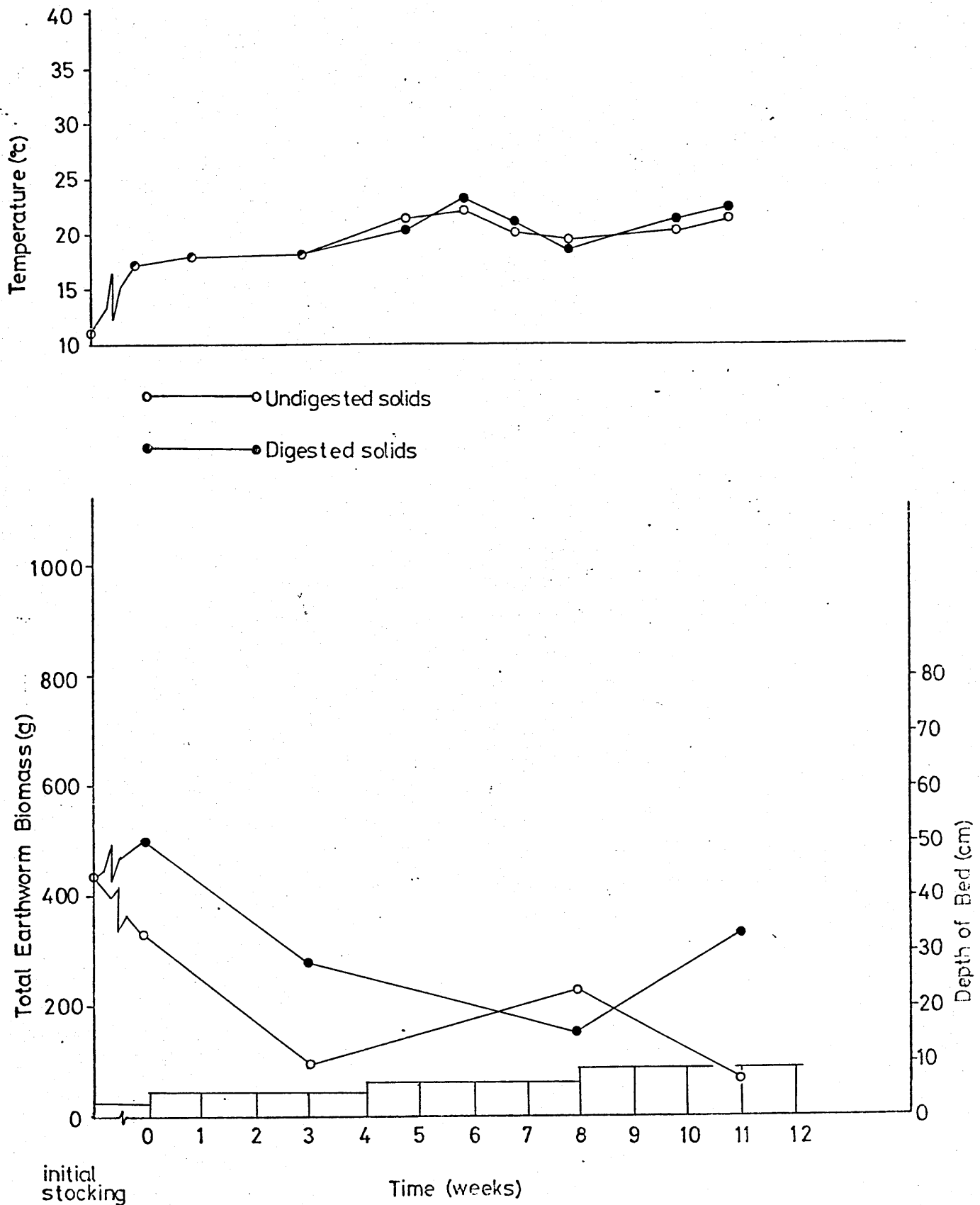
2.2.4) Sampling.

To sample the initially shallow beds a 25 by 25cm quadrat was used, and randomly placed on the solids of the bed. The solids and earthworms enclosed within the quadrat were taken up and the earthworms hand-sorted from the compost. The earthworms were then washed in water and dried on a paper towel prior to weighing. As the experiment proceeded and more solids were added to the beds increasing their depth, a core of 10.3cm internal diameter was used in the same way as the quadrat for sampling. Three samples per bed were taken for the quadrat and five for the core, the difference arising from the relative areas sampled by each method.

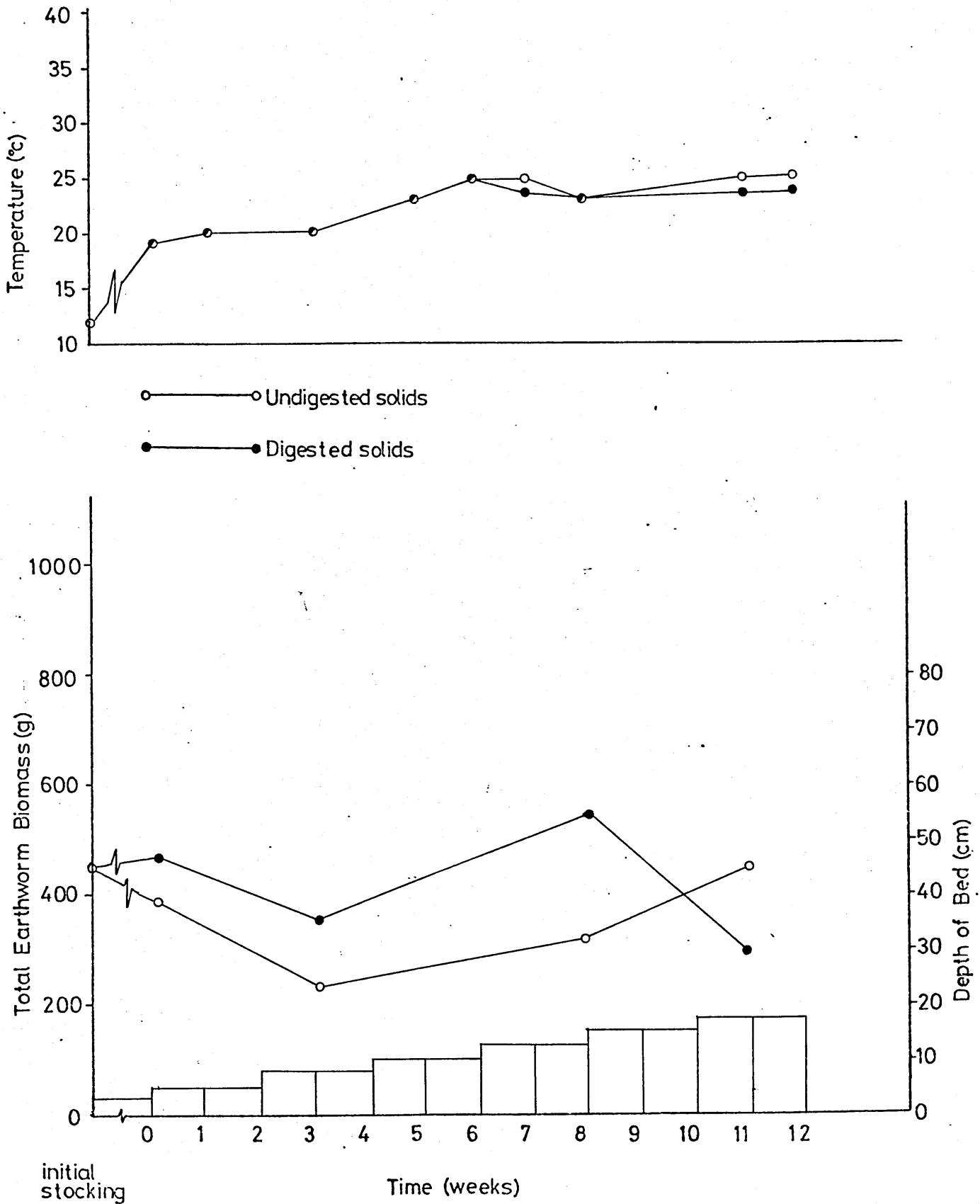
2.2.5) Results.

Graphs 2.2.5.1 to 2.2.5.5 show changes in the total earthworm biomass and average individual weight on the increase in bed depth, recorded as a histogram. Also presented is the average bed temperature at a depth of 10cm recorded over the course of the experiment. Graph 2.2.5.6 shows the average size distribution of earthworm populations at the end of the experiment.

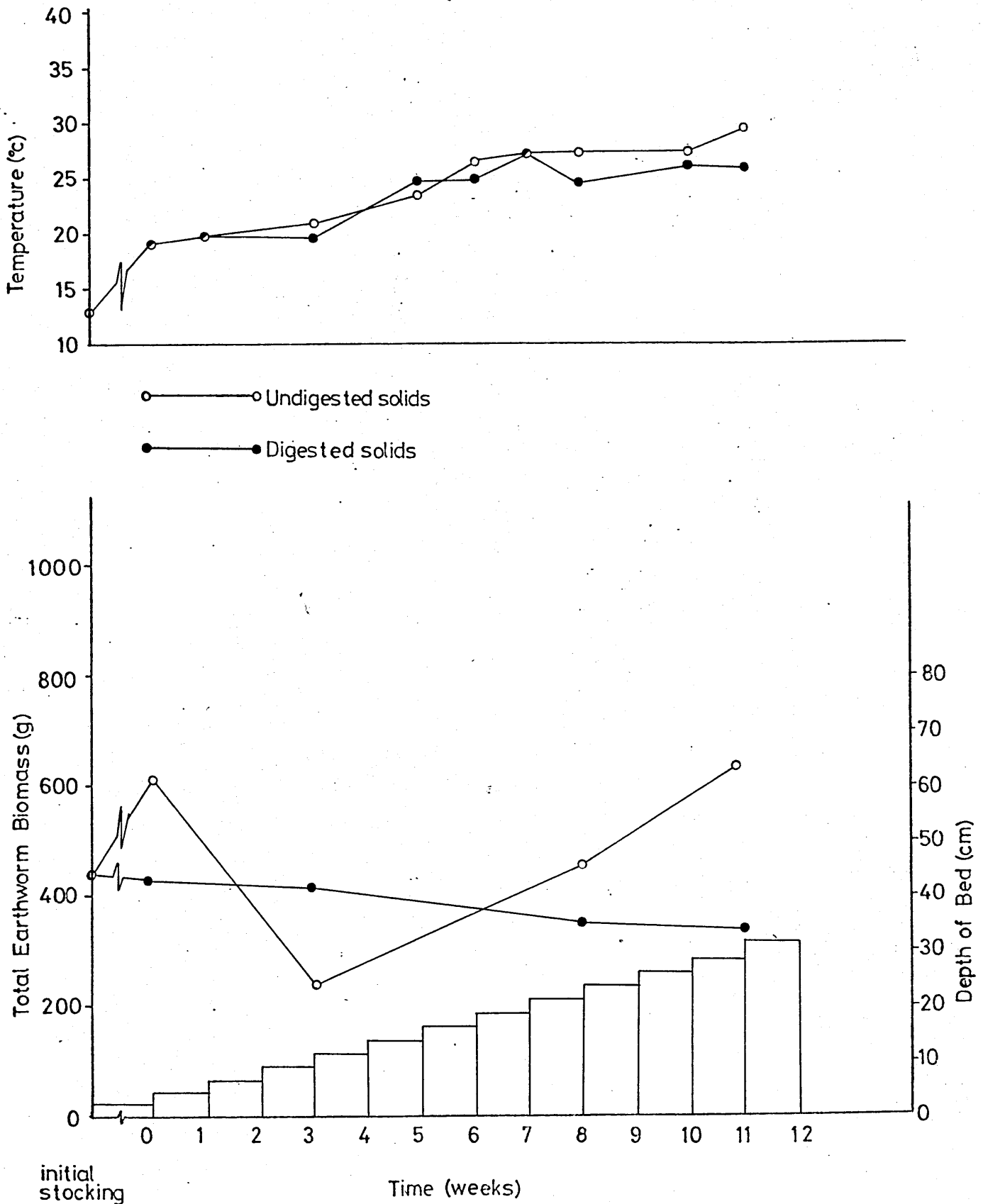
Graph 2.25.1. Change in Earthworm Biomass and Temperature on the Addition of Extra Solids. Treatment 1.



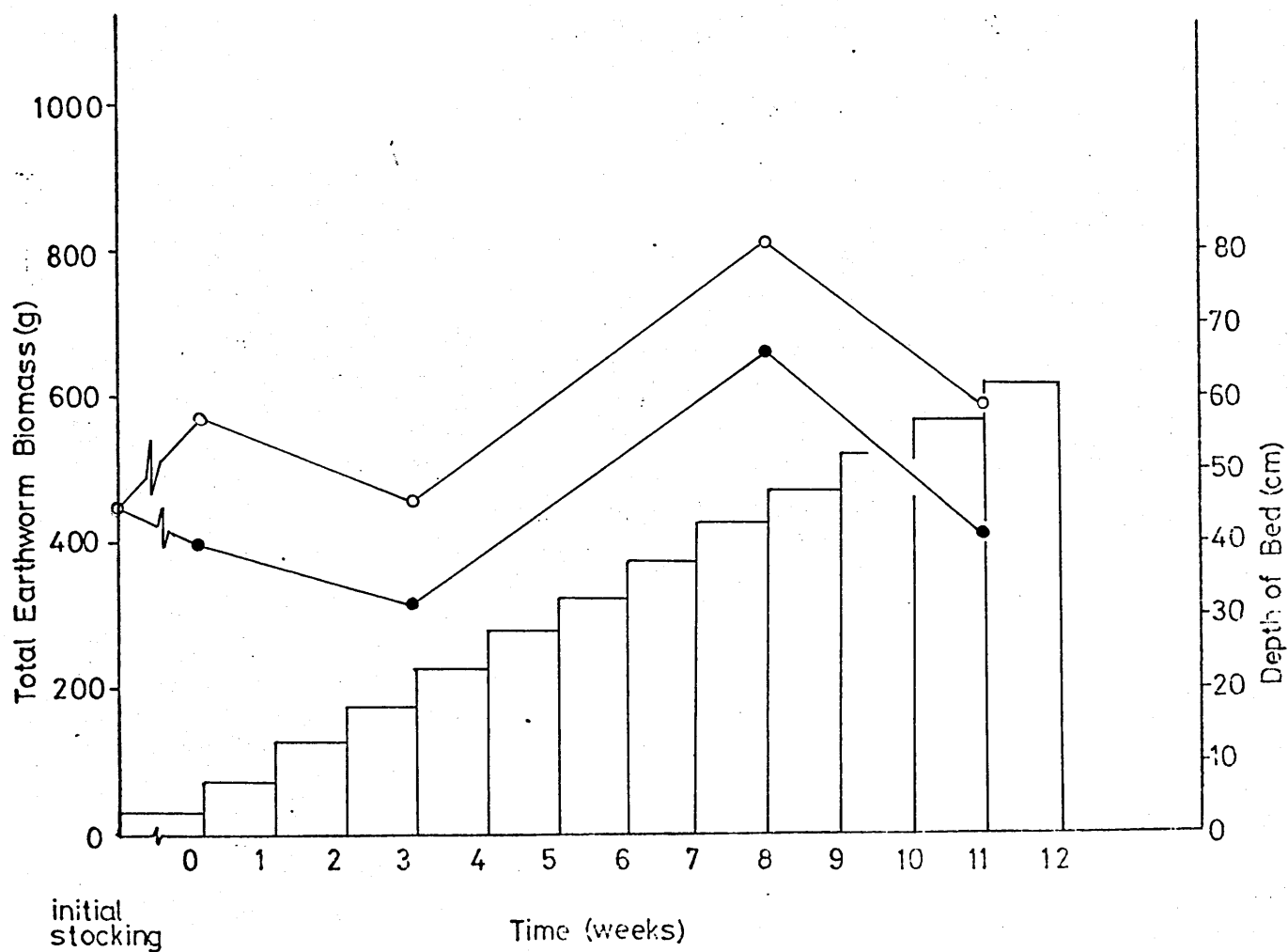
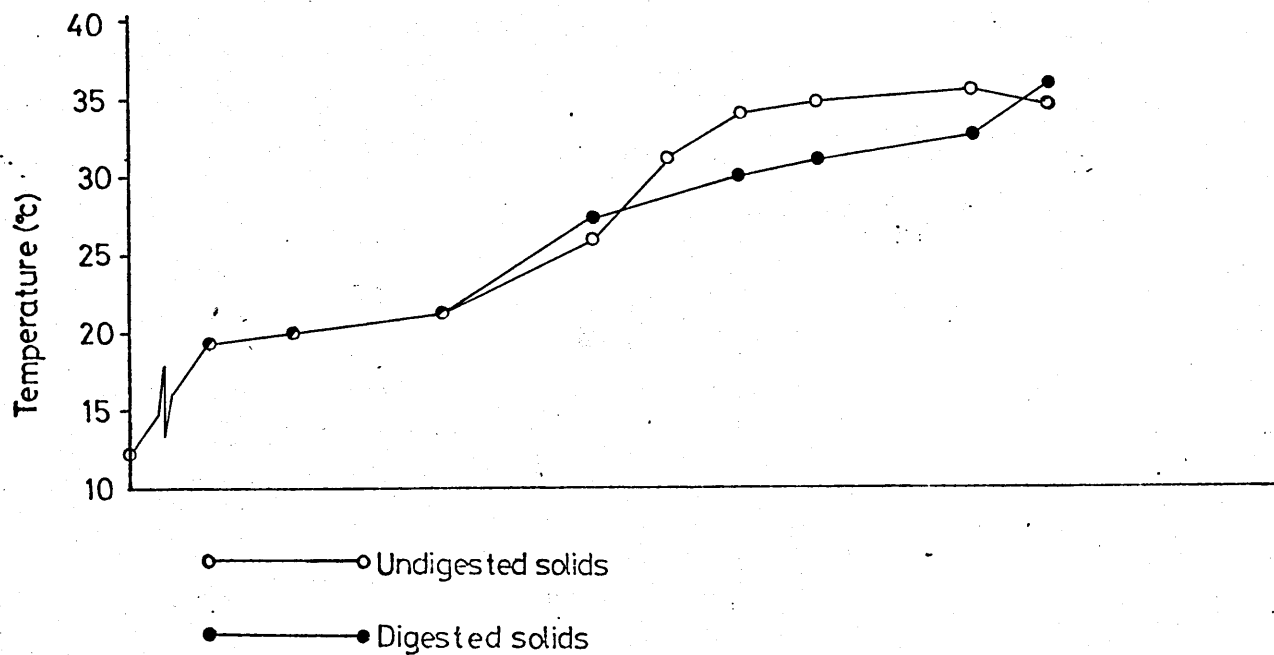
Graph 2.252. Change in Earthworm Biomass and Temperature on the Addition of Extra Solids. Treatment 2.



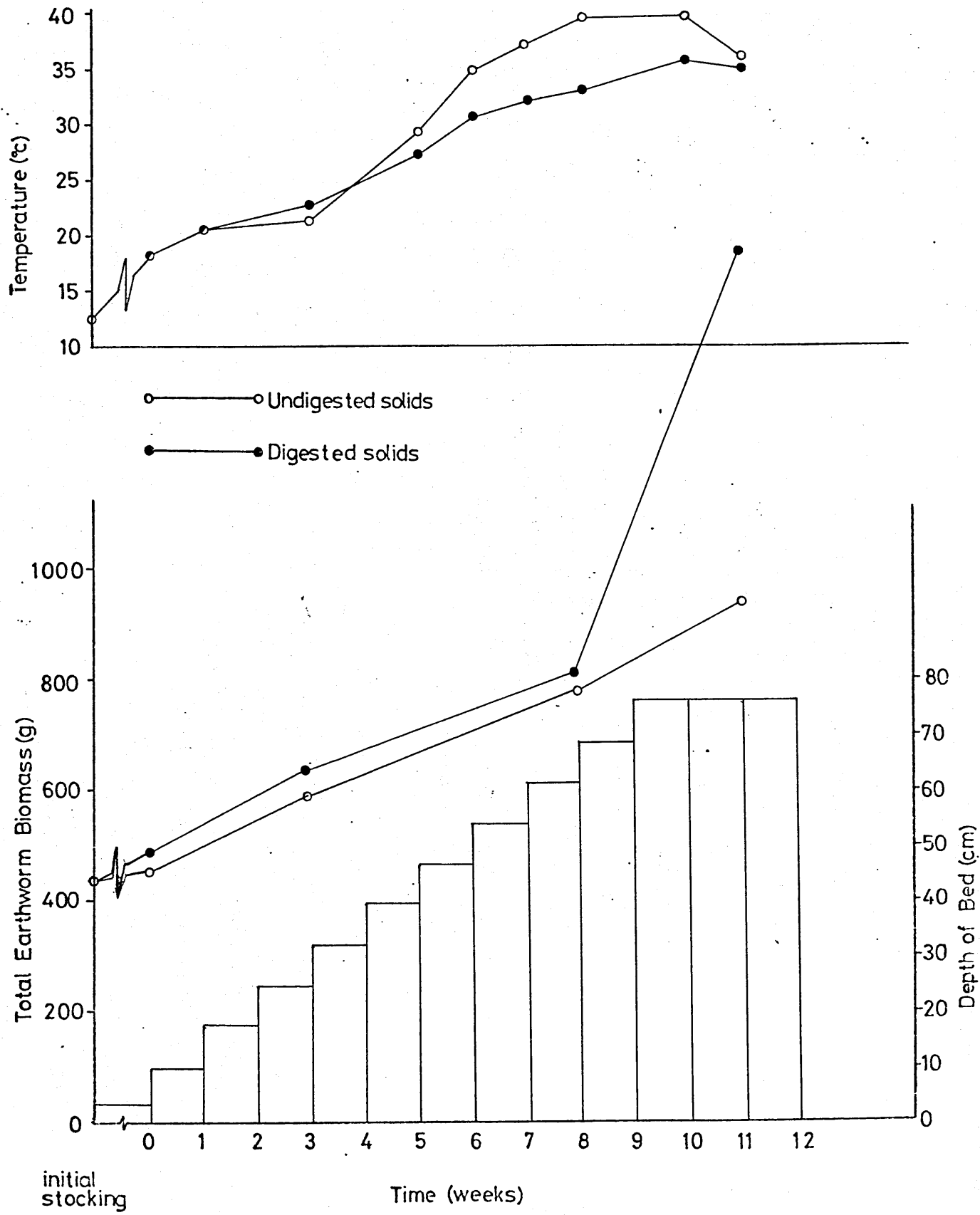
Graph 225.3. Change in Earthworm Biomass and Temperature on the Addition of Extra Solids. Treatment 3.



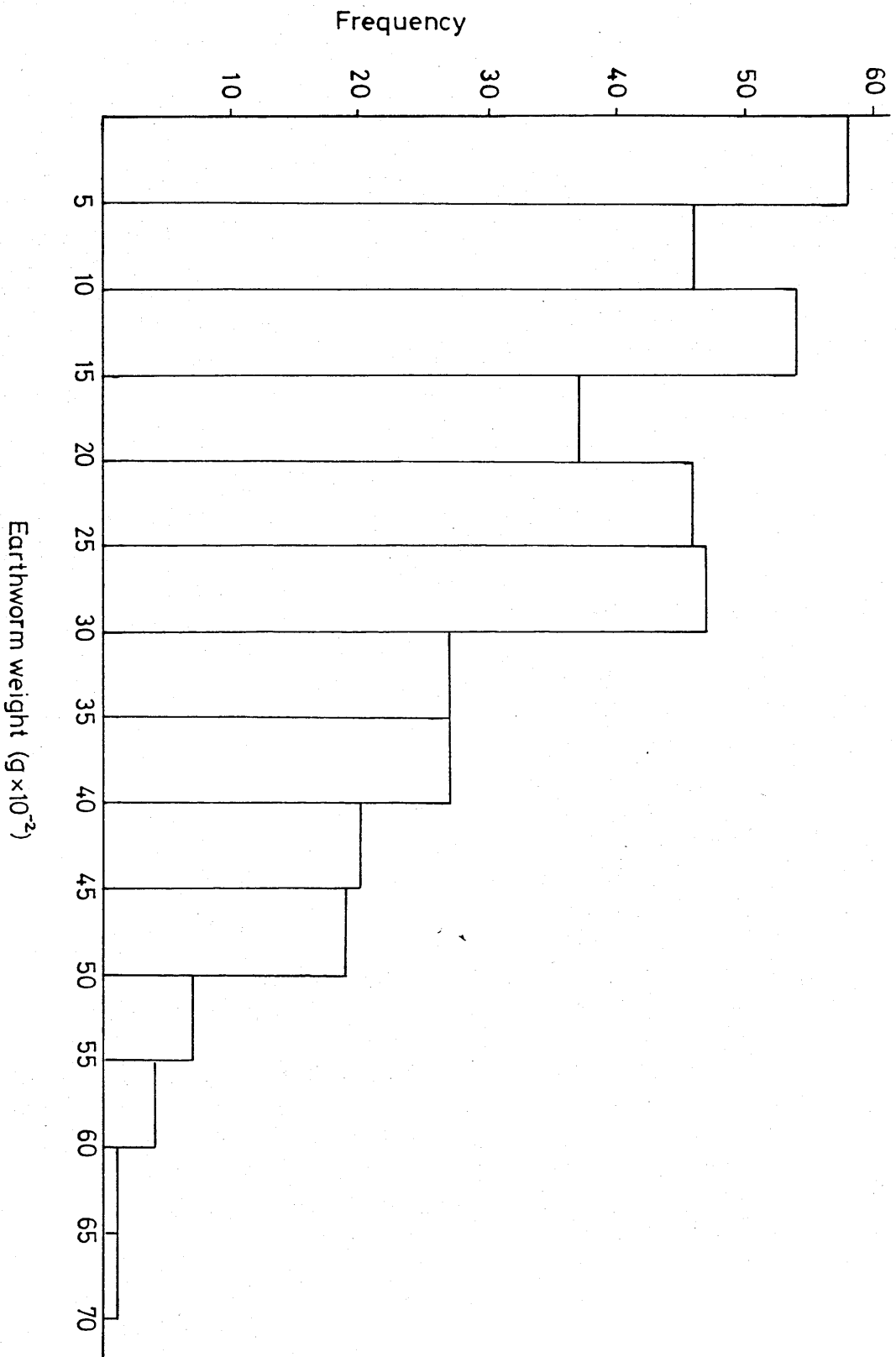
Graph 2254. Change in Earthworm Biomass and Temperature on the Addition of Extra Solids. Treatment 4.



Graph 2.255. Change in Earthworm Biomass and Temperature on the Addition of Extra Solids. Treatment 5.



Graph 2.2.5.6. Size Distribution of Earthworms Sampled from
Bore Place Beds



2.2.6) Discussion of Results.

The results presented in graphs 2.2.5.1 to 2.2.5.5 show that there was only an increase in earthworm biomass at the highest solids toploading rate, treatment 5. Use of Students t-test shows that this was the only statistically different final earthworm biomass comparing all solids addition rates and digested and undigested material. Even here there was a long lag phase before any biomass increase occurred, considering the initial period of 8 weeks between inoculation and the start of the experiment. This period should have been sufficient for the production of cocoons in all treatments. Cocoons take a minimum of 4 weeks to hatch at 20°C (Tsukamoto and Watanabe 1977), but in sub-optimal conditions this could be lengthened considerably. Evans and Guild (1948) observed an 11 week hatching time for *E.fetida* cocoons in spring. The hypothesis that earthworm numbers increase in line with the increased amount of waste available is shown not to be the case

Once hatched the earthworms require a minimum of seven weeks to become sexually mature. The very low biomass increases at the highest solids loading rate would indicate that there was no cocoon production when solids loading began.

If this is so, then only one new generation of earthworms would be possible in the time available. The size distribution of earthworms recorded at the end of the experiment (graph 2.2.5.6) shows a very high proportion of hatchlings and smaller earthworms, indicating that the increase in treatment 5 were due to new individuals entering the population.

At the end of the experiment a total of 583kg of undigested and digested solids had been added to treatment 5. The maximum recorded weights of earthworm biomass for the two beds, 900 and 1600g respectively, represent conversion efficiencies of 0.15 and 0.27% wet weight to wet weight, which is low compared to conversion efficiencies of 5% from

laboratory data (Neuhauser *et al* 1980).

The amount of material added was clearly not a limiting factor to earthworm population growth. This suggests that conditions within the solids were not optimum in some way, either through an environmental factor such as temperature or moisture content, or some physico-chemical factor. The effect was to inhibit both the growth of earthworms in the original inoculum, and also to inhibit reproduction to produce the second generation.

Visual inspection of the base of the beds at higher top-loading rates revealed an accumulation of water from the mass of solids above. This indicated poor drainage, and suggests that the drainage system designed for the earthworm beds is not adequate to cope with the free water being produced from the high rates of solid toploading. Some discolouration of the waste was observed and malodours were detected at the base of the core samples that were taken. A distinct zonation of earthworm distribution was also noted from the core samples, with more individuals found in the upper layers. However, it is also possible that this may be due to movement out of the worked solids into fresh material being loaded onto the beds.

If anoxic conditions were present in the base of high loading rate beds, and so making them toxic to earthworms, this may account for the very low conversion ratios because only a small proportion of the total mass of solids is available as a food source.

Another important feature of the results is the high temperatures recorded from the solids in treatment 5, 40 and 36°C respectively. From the literature such temperature are high enough to cause mortalities to *E. fetida* (Hartenstein 1982) and may account for the low increase in earthworm population biomass. In beds with higher temperature there was a noticeable cluster of earthworms towards the edges of the beds. This was almost certainly a thermotactic response, as documented by Wolf (1940), and one effect of this would be to reduce the apparent population size as measured by sampling, because not

enough samples could be taken to overcome the non-random distribution of earthworms.

It is interesting to note a correlation between temperature increase and the rate of solids loading. This would indicate that as the mass of solids within the bed increases the rate of heat production becomes greater than the rate of heat loss. Increased heat production may also arise through an increased rate of decomposition, initiated by the burrowing and feeding activities of the earthworms present, increasing aerobic metabolic processes and so releasing heat. The extra mass of solid within the bed will increase its insulation and so cause the temperature to rise. If this is so then the higher temperatures recorded for undigested solids are to be expected, as many of the energy rich molecules in digested solids will have been broken down on anaerobic digestion (Stafford *et al* 1980). The greater earthworm biomass in digested solids in treatment 5 may reflect the fact that lower temperature induced mortalities were occurring.

In summary, only in the highest top loading rate was there any increase in earthworm population, although the size of the increase may have been hidden by sampling problems caused by the uneven spread of earthworms at high temperatures. The temperatures recorded in treatments 3, 4 and 5 may have inhibited population increase, and anaerobic conditions may have been created in the lower levels of beds, especially for undigested solids with a high moisture content. Unfortunately, given the farm situation no detailed physico-chemical analyses of the solids could be carried out.

There was no difference between earthworms grown in undigested and digested solids, except in treatment 5 when at the end of the experiment the total earthworm weight in digested solids was almost double that in undigested solids. It is probable that this is a temperature effect rather than a response to different physico-chemical factors. These results indicate that further experiments with digested solids are worthwhile.

2.3) The Effect of Bed Depth on Earthworm Populations.

2.3.1) Introduction.

The hypothesis presented in section 2.2 was shown not to hold, so that increasing the amounts of solids available for earthworms did not lead to a corresponding increase in earthworm population. This indicates that other factors have become involved, limiting earthworm growth below a theoretical maxima. Two possible factors are suggested.

Firstly increased rates of decomposition generated metabolic heat which was not released because of the large amount of material present causing an increase in temperature. Secondly because of poor drainage from the beds water may have accumulated at the base, reducing air spaces within the solid, exacerbated by compaction and so creating pockets of anaerobiosis.

In both cases, the deeper the bed became, the greater the problem. In this experiment the effects of bed depth on earthworm populations is studied, keeping all other factors within the experiment constant, apart from the amount of solids within each bed. Undigested solids were used for this experiment, and an earthworm stocking density of 100/1 wet weight to wet weight was used in each treatment. This produces a range of different earthworm numbers in the treatments, but it was felt better to standardise the ratio of earthworms to waste rather than absolute numbers. The experimental layout was as follows;

Treatment 1	Bed depth 20cm
Treatment 2	Bed depth 30cm
Treatment 3	Bed depth 40cm
Treatment 4	Bed depth 50cm
Treatment 5	Bed depth 60cm

2.3.2) Materials And Methods.

The beds were loaded with freshly separated undigested solids to the required depth and allowed to become acceptable to earthworms. This process was aided by hand turning the beds weekly to keep them aerobic and increase the rate of ammonia volatilisation. This was carried out in the summer and the higher ambient temperatures compared to the previous experiment meant that the material was acceptable in three weeks. During this time there was a degree of settling within the beds, and so extra undigested solids were added to increase the depth of the beds to the required value.

2.3.3) Inoculation.

The inoculum was a commercially cultured population of *E.fetida* obtained from British Groundbaits. Prior to inoculation the earthworms were stored in peat in hessian bags at 10°C. Graph 2.3.4.1 shows the size distribution of the earthworm population prior to inoculation.

After composting and settling the bulk density of the solids had increased from 773 to 997kg/m³. Therefore, for every 10cm depth of the 1m² beds there was approximately 100kg of solids. Based on a 100/1 wet weight to wet weight inoculation rate the following weights of earthworms were used;

Treatment	Bed Depth	Weight of Solids	Earthworm
	(cm)	(kg)	Inoculum (kg)
1	20	200	2
2	30	300	3
3	40	400	4
4	50	500	5
5	60	600	6

The earthworm/peat mix was spread evenly over the surface of each experimental treatment.

2.3.4) Results.

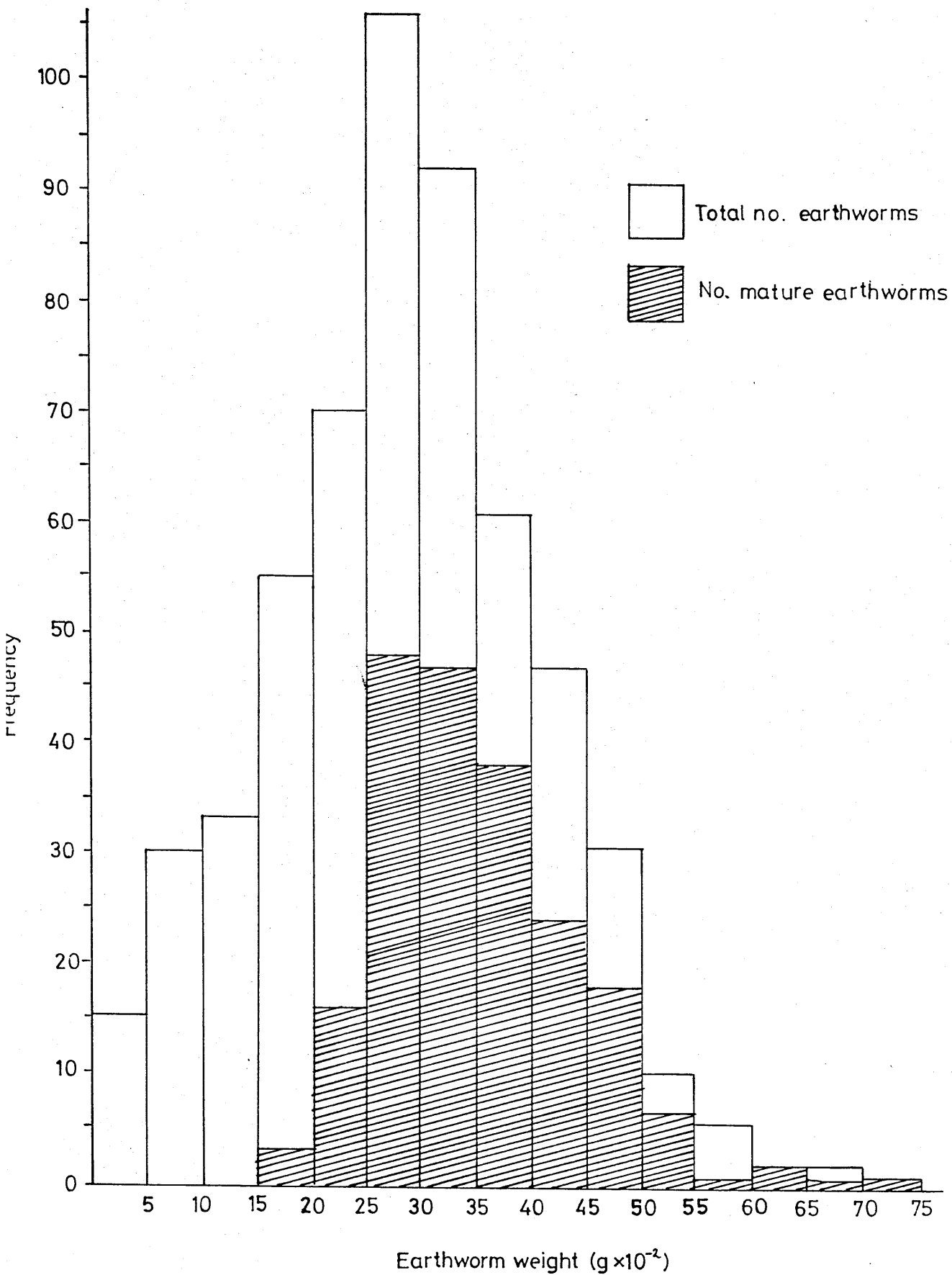
Graph 2.3.4.1 shows the class distribution of the *E.fetida* population used as an inoculum. Table 2.3.4.1 shows the change in total weight, average weight, percentage mature and number of cocoons produced by the earthworm population in the various treatments. Graph 2.3.4.2 plots the earthworm to solids weight ratio in each treatment against time, and graph 2.3.4.3 gives the temperature regime in the earthworm tunnel prior to inoculation

Table 2.3.4.1.

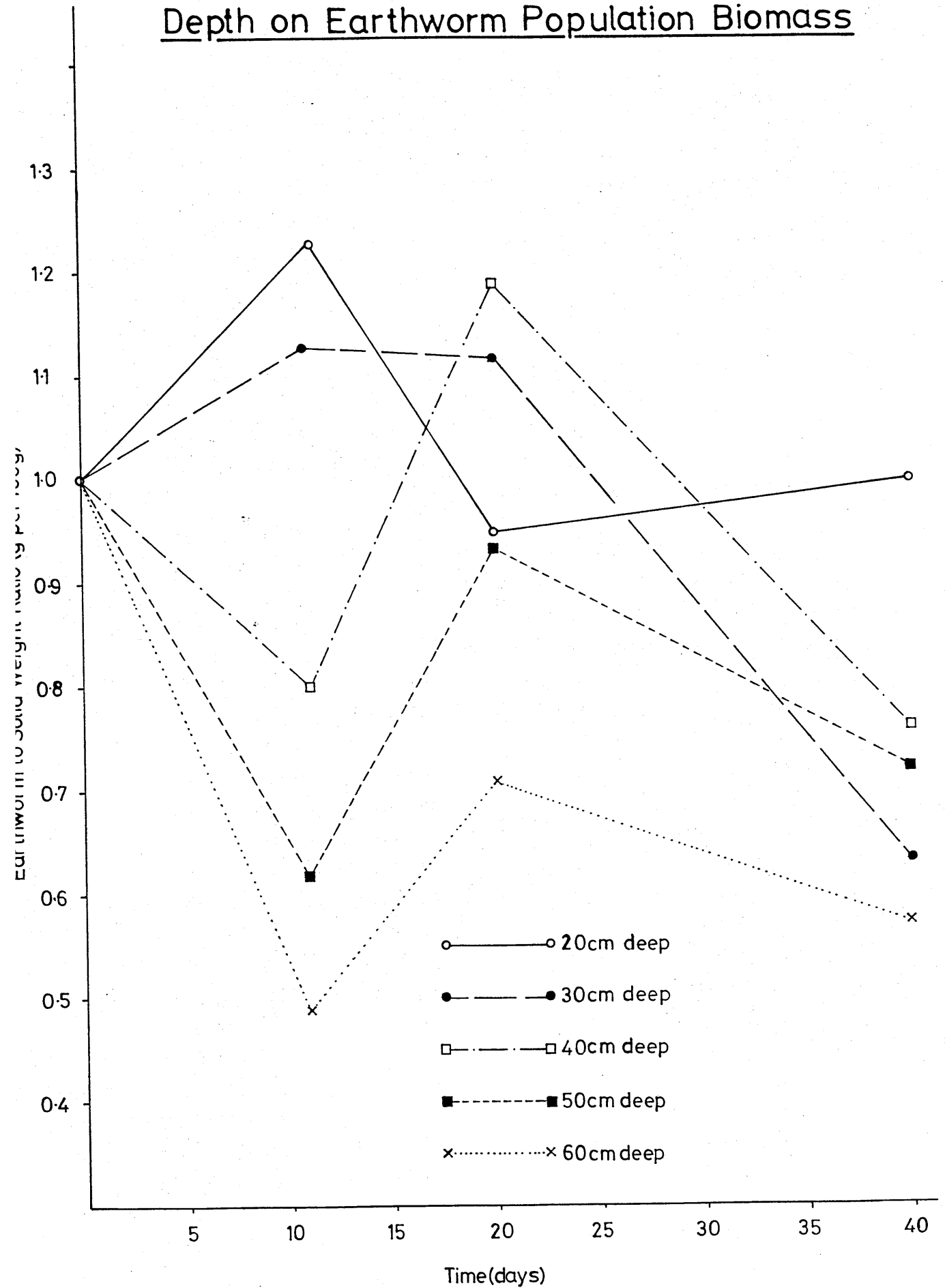
Earthworm Population Dynamics at Various Bed Depths.

Day	0	12	20	40
Treatment 1. Bed 20cm deep				
Total Wt. (g)	2000	2463	1925	1982
Av. Wt. (g)	0.29	0.45	0.42	0.3
% mature	36	47	40	47
No. cocoons	0	1052	2632	5300
% nos. <0.15g	13.9	8.7	8.4	2.1
Treatment 2. Bed 30cm deep.				
Total Wt. (g)	3000	3433	3349	1926
Av. Wt. (g)	0.29	0.40	0.44	0.35
% mature	36	42	42	43
No. cocoons	0	913	2703	6213
% nos. <0.15g	13.9	14	4.6	8.8
Treatment 3. Bed 40cm deep.				
Total Wt. (g)	4000	3215	4776	3051
Av. Wt. (g)	0.29	0.39	0.38	0.37
% mature	36	39	36	53
No. cocoons	0	667	2738	6423
% nos. <0.15g	13.9	6.8	6.4	1.7
Treatment 4. Bed 50cm deep.				
Total Wt. (g)	5000	3074	4693	3565
Av. Wt. (g)	0.29	0.38	0.40	0.31
% mature	36	43	47	48
No. cocoons	0	772	1720	6950
% nos. <0.15g	13.9	11.3	6.0	12.23
Treatment 5. Bed 60cm deep.				
Total Wt. (g)	6000	2968	4275	3419
Av. Wt. (g)	0.29	0.32	0.35	0.30
% mature	30	33	43	35
No. cocoons	0	350	1123	3264
% nos. <0.15g	13.9	17.4	9.9	16.0

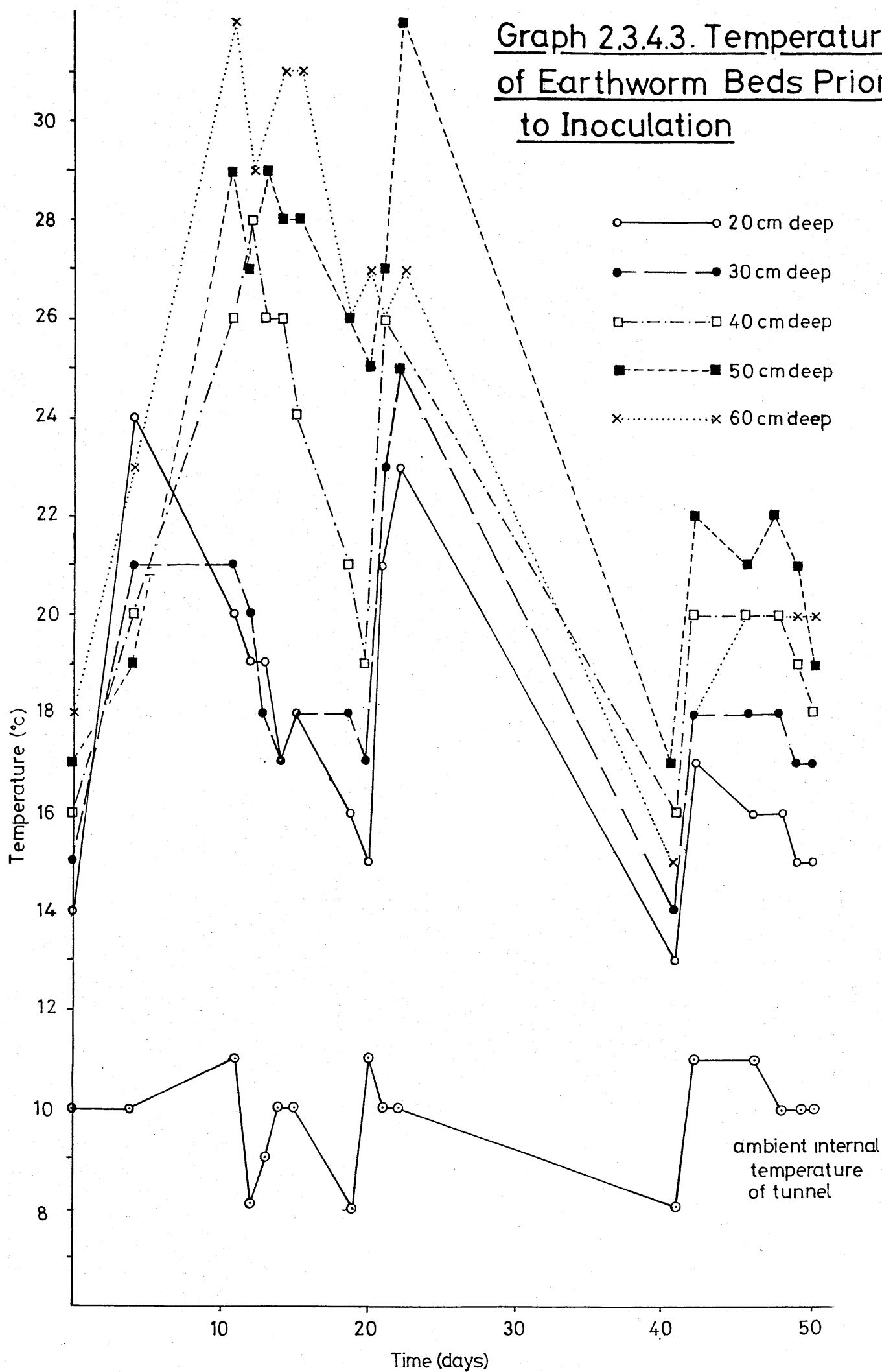
Graph 2.3.4.1. Size Class Distribution of Earthworm Population used as an Inoculum



Graph 2.3.4.2. The Effect of Changing Bed
Depth on Earthworm Population Biomass



Graph 2.3.4.3. Temperature of Earthworm Beds Prior to Inoculation



2.3.5) Discussion of Results.

The results show very little weight increase in any of the treatments although there is a slight rise above the initial rate for treatments 1, 2, and 3, but all had fallen below the weight of the inoculum by the end of the experiment. Treatments 4 and 5 never increased above the initial inoculum weight.

There is a steady increase in cocoon numbers in all treatments during the course of the experiment, but the earthworm population in the deepest bed seems to have suffered reproductive inhibition, with a reduced percentage of mature earthworms in the bed, and reduced cocoon production compared to other treatments. This would indicate that the high proportion of earthworms weighing less than 0.15g in treatment 5 at the end of the experiment occurred through a regression in size rather than the production of new individuals.

In section 2.2 temperature was considered an important variable effecting earthworms in deeper beds. Table 2.3.4.2. shows the temperature recorded in this experiment. The maximum increase in temperature occurred in the first 13 days after inoculation. Such a result is consistent with the earlier experiment, in that the introduction of earthworms to the solids appears to have stimulated microbial decompositional activity by aerating and macerating the solids, introducing oxygen and increasing the surface area. However even in the deepest beds the temperature only reached a maximum of 33°C compared to 40°C recorded in the earlier section. This included a period of undercoil heating to raise the temperature of other beds.

The temperatures recorded in this section were not as high as those in section 2.2 for two reasons. Firstly, the maximum bed depth reached at the highest top-loading rate in section 2.2 was 77cm compared with 60cm in this trial. Secondly the experiment was carried out in winter when the air temperature within the earthworm tunnel was lower

than previously experienced. In the period 1st April '82 to 17th September '82 the mean maximum/minimum air temperatures in the earthworm tunnel were 19.7/14.4°C, compared to 11.4/8.7°C for the period 26th Nov '82 to 13th Jan '83.

The temperature reached in the 50 and 60cm deep beds, between 28 and 33°C were high enough to cause mortalities according to the literature (Hartenstein 1982, Kaplan 1980) accounting in part for the population decreases. In addition, Hartenstein (1982) notes that temperature rises above 28°C inhibit sexual maturity occurring in *E.fetida* whereas temperatures below the optimum only increase the time taken for the earthworm to become sexually mature. Such inhibition could account for the reduction of sexual maturity and cocoon production observed in treatment 5.

However, there was also little increase in growth in the more shallow beds, and in these treatments the temperature rarely rose above 25°C. The lower temperature recorded in treatments 1 and 2, averaging 18°C may have been within the inhibitory temperature band as described by Hartenstein (1982), reducing growth and reproduction rates. Only treatment 3, with an average temperature of 20°C was within the quoted optimum temperature range, and here a slight increase in population size was observed during the course of the experiment.

The results indicate that earthworm populations are responding to the temperature regimes in the earthworm beds, but such responses are being obscured by some factor which is tending to inhibit the growth of earthworms in all treatments. Since gross physical parameters were in accordance with the requirements for earthworm growth it is likely that one, or a combination of physico-chemical factors is having a general inhibition effect on earthworm growth. It is impossible to speculate at this stage what physico-chemical factors are involved.

Thus even though there is a general masking effect, the results obtained do indicate that

the depth of an earthworm bed is an important consideration when planning field scale earthworm culture. The temperature differences that occur on changing bed depth can be theoretically shown to influence earthworm populations, even if the growth response of earthworms in this experiment was poor. From the results obtained, a bed depth of 40cm produces the optimum temperature regime in these particular circumstances, but this cannot be taken as a general value that will hold in all situations.

2.4) The Effect of Varying the Stocking Density on Earthworm Populations.

2.4.1) Introduction.

In section 2.3 all factors effecting the experiment were kept as constant as possible apart from the bed depth, and thus the amount of solid material in each bed. The ratio of earthworms to solids was standardised at a stocking density of 100/1 wet weight to wet weight, but this produced varying numbers of earthworms per bed which may have effected the results. In the following experiment bed depth is kept constant and the stocking density altered. The hypothesis is that the initial stocking density of an earthworm bed is important to the subsequent growth and development of the population. Although in theory the initial stocking density should not effect the maximum earthworm biomass achieved in an earthworm bed, the time taken to reach this maximum, and the rate at which the cattle solids are broken down are both dependent on the initial stocking density.

2.4.2) Experimental Layout.

A fixed amount of fresh separated undigested solids were placed in 1m² beds to a depth of 30cm, and allowed to become acceptable to earthworms. Once acceptable varying weights of earthworms were added to the beds to produce five different stocking densities. The weight of undigested solids in the beds at 30cm depth was 299kg, and so the weight of *E.fetida* required to produce varying stocking density rates was as follows;

Treatment	Stocking Density (wet wt/wet wt)	Earthworm wt. (kg)
1	200:1	1.49
2	100:1	2.90
3	80:1	3.74
4	60:1	4.99
5	40:1	7.48

2.4.3) Results.

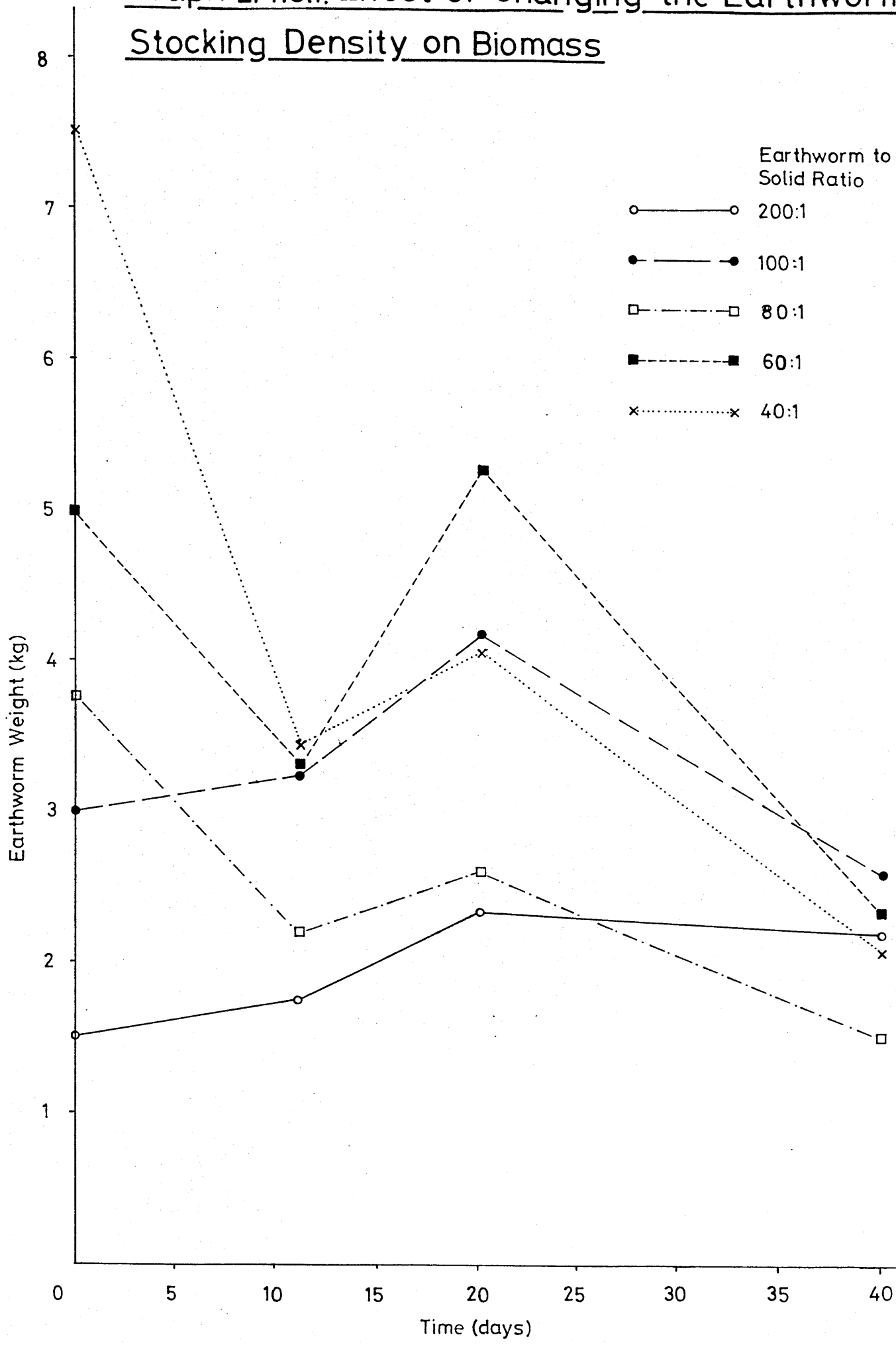
The size class distribution of the inoculum is the same as that shown in graph 2.3.4.1. Table 2.4.3.1 shows the total weight, average weight, percentage mature and cocoon production for earthworms in the various treatments. Graph 2.4.3.1 plots the change in total earthworm biomass over time for the 5 treatments. The temperatures of the earthworm beds were measured over the course of the experiment and found to vary similarly in all treatments, with maxima and minima of 26°C and 14°C respectively. The ambient temperature of the earthworm tunnel was as in graph 2.3.4.3.

Table 2.4.3.1.

Earthworm Population Dynamics at Five Stocking Densities

Day	0	12	20	40
Treatment 1. Stocking Density 200/1.				
Total Wt. (g)	1490	1738	2350	2201
Av. Wt. (g)	0.29	0.45	0.44	0.44
% mature	36	39	55	70
No. cocoons	0	160	1578	4272
% nos. <0.15g	13.9	5.0	4.0	1.4
Treatment 2. Stocking Density 300/1.				
Total Wt. (g)	2990	3224	4157	2620
Av. Wt. (g)	0.29	0.43	0.44	0.38
% mature	36	45	49	56
No. cocoons	0	805	2069	8312
% nos. <0.15g	13.9	1.9	3.4	2.0
Treatment 3. Stocking Density 400/1.				
Total Wt. (g)	3740	2226	2635	1499
Av. Wt. (g)	0.29	0.34	0.35	0.36
% mature	36	27	25	46
No. cocoons	0	210	666	1499
% nos. <0.15g	13.9	9.4	12.0	1.9
Treatment 4. Stocking Density 500/1.				
Total Wt. (g)	4990	3325	5284	2322
Av. Wt. (g)	0.29	0.42	0.42	0.34
% mature	36	48	38	53
No. cocoons	0	491	1894	2630
% nos. <0.15g	13.9	11.0	3.3	7.6
Treatment 5. Stocking Density 600/1.				
Total Wt. (g)	7480	3395	4100	2081
Av. Wt. (g)	0.29	0.36	0.35	0.32
% mature	34	37	25	18
No. cocoons	0	456	491	666
% nos. <0.15g	13.9	9.9	7.3	7.0

Graph 2.4.3.1. Effect of Changing the Earthworm
Stocking Density on Biomass



2.4.4) Discussion of Results.

Only in treatments 1 and 2 does the total earthworm biomass increase slightly over the 40 day period of the experiment. In all other treatments the earthworm population lost weight. Taking a theoretical 10% conversion efficiency, as recorded by Spedding *et al* (1981), then a possible maximum biomass of 30kg could have been achieved. the highest recorded figure was the 7.5kg used as the initial inoculum in treatment 5 which dropped immediately.

The results show high cocoon counts in treatments 1 and 2, with an increase in the percentage of mature earthworms to over 50%, and with a corresponding decrease in earthworms weighing less than 0.15g. However, the average earthworm weight remained around 0.44g, indicating that for the stocking densities of 200:1 and 100:1 the earthworms have diverted their energy into cocoon production rather than weight increase. This may have been an 'insurance' response to produce more cocoons which are more likely to survive a period of adverse conditions than juvenile or adult earthworms. Lee (1959) investigated populations of *Alonga* in saline soils prone to desiccation and found no earthworms survived the summer period, but the population regenerated each year from cocoons laid the previous spring. Bouche (1977) defines such a strategy for earthworms living in unstable environments such as the litter layer of soil. In treatments 3, 4 and 5 the average earthworm weight was lower than in treatments 1 and 2, with fewer cocoons produced and a higher percentage of earthworms weighing less than 0.15g. This suggests that at these higher stocking densities earthworms were competing with each other for limited resources, and so losing weight. However, even at the higher stocking densities one would theoretically expect there to be enough organic material to allow some increase in the earthworm population.

Overall the results show that under the conditions of this experiment the Bore Place undigested separated solids do not support earthworm growth as well as similar animal

excreta cited in the literature (Edwards *et al* 1985, Neuhauser *et al* 1980), and as in section 2.3, the effect of variation in stocking density on earthworm populations has been obscured by the lack of growth. There are at least two possible explanations of the results; either the separated solids do not offer enough nutritional value, or environmental or physico-chemical factors are sub-optimal for earthworms, either disrupting normal feeding patterns, inhibiting growth and/or reproduction or actually causing earthworm mortalities.

The results show that the earthworm populations at lower stocking densities (200:1 and 100:1) can produce small population size increases, but the separated solids cannot support higher numbers of earthworms, leading to a fall in biomass at densities above 80:1. In fact from graph 2.4.3.1, all of the final population weights are grouped around 1.5 to 2.5kg, indicating that this weight of earthworms is about the maximum that can be supported in a 1m² bed filled to a depth of 30cm.

2.5) The Effect of Different Pretreatment Regimes upon Separated Solids Prior to Inoculation with Earthworms.

2.5.1) Introduction.

The limited earthworm population biomass gain observed in sections 2.2, 2.3, and 2.4 has been attributed either to a lack of a nutritional factor within the waste, or sub-optimal environmental conditions which have adversely affected the development of earthworms.

Whichever of these two factors are involved (or a combination of the two) there are two possible ways in which such conditions may have arisen. Firstly there may be an inherent feature of the diet of the cattle at Bore Place, varying with the constituents used to make up the feed of the cattle, their age and the environment in which they are kept. Secondly, the way in which the cattle slurry is treated after it has been produced will have important implications for the nutritional and physico-chemical factors occurring within it. This involves the storage of the slurry prior to separation, the separation process itself and preparing the separated solid prior to inoculation with earthworms.

In a working farm the diet and environment of the cattle are impossible to alter experimentally. Also the storage facilities and the time of storage are controlled by practical criteria, and again not within the sphere of experimental manipulation. The separator also has little flexibility for change once set up.

However, the way in which the solids are pretreated can be experimentally altered, and is perhaps the most important process affecting earthworms, as the greatest number of changes within the waste occur at this juncture, in the progression from an anaerobic to aerobic state.

The following experiment was therefore initiated to test several ways of pretreating separated cattle solids in order to gain a better understanding of the mechanisms by which separated cattle solids change from an unacceptable to acceptable state for earthworms, and to ascertain if it is this process being carried out inefficiently which is causing the generally poor results as observed in earlier experiments.

Hand-turning and forced aeration are adopted as two methods of aerating and mixing agricultural wastes. Aeration has been used with some success in the treatment of sewage sludges (Parr *et al* 1978). The experiment therefore has the secondary aim of examining the feasibility of this method of solid pretreatment for possible utilisation on a large scale as an alternative to mechanical methods of mixing and aerating solids.

2.5.2) Experimental Design.

Both raw and digested solids were used in this experiment, and pretreated in one of two ways;

Hand Turning;

The solids were turned by forks, so that the top layers of solid were buried by solids from the lower layers. Hand turning was carried out once per week.

Forced Aeration;

A Handyair no. 2 compressor delivering 30 litres per minute was used to pump air through 1cm bore piping with 2mm holes drilled every 20cm and facing downwards to avoid blockage from particles within the solids. The pump was run for 8 hours per day for the first 9 days until the temperature reached 39°C. After this point the pump was activated when the temperature levelled off or fell.

The experiment was arranged thus;

Treatment	Type of Solid	Method of Pretreatment
1	digested	hand turning
2	digested	forced aeration
3	undigested	hand turning
4	digested	hand turning (control, no earthworms)

Some of the marine ply boarding dividing the earthworm bed into 1m² bays were removed to create 4 bays of 2m². The bay layout was as follows;

Bed no.	Type of Material	Pretreatment
1	Digested solids	Hand-turned
2	Digested Solids	Aerated
3	Digested Solids	Hand-turned
4	Undigested solids	Hand-turned
5	Inoculum	Control

Prior to inoculation the material in the bed was heaped into piles to maintain a critical mass for heat retention during the pre-treatment process, which was carried out over a period of 28 days. When pre-treatment was complete the material was spread out over the base of the bed and the heat within it was dissipated. 6 days later the beds were inoculated with earthworms.

2.5.3) Inoculation.

A mixture of earthworms from Bore Place and Rothamsted Experimental Station were used for the inoculation. The solids in beds 2, 3 and 4 were piled to one side of the bay. A proportion of Bore Place inoculum was placed in the bay, along with 2 sacks of Rothamsted inoculum. The solids in the bed were then piled on top of the inoculum already in place, and the other half of the inoculum placed at the bottom of the bed,

and the solids then evened out so that the earthworm inoculum was at the base of the bed for each treatment. A layer of hessian sacking was then placed over each of the experimental beds to retain moisture in the upper layers of the solids but also allow gaseous diffusion. Previous trials had shown a degree of drying of the surface layers, several centimeters deep.

2.5.4) Physico-Chemical Analyses.

The solids in this experiment were tested for pH, ammonia and ammonium ion concentration, total solids and ionic conductivity using the following methods;

pH.

Measured directly from the solids with an EIL probe and Digital Water Analyser.

Total solids (T.S.%).

The material is weighed, dried overnight in an oven set at 105°C, cooled in a desiccator and reweighed. the percentage total solids is measured by difference.

Ammonia and ammonium ions.

The concentration of the above was measured with an NH_3 probe connected to a Digital Water Analyser. Samples were measured as an aqueous extract, 4g fresh weight of solids in 20ml of distilled water. Free ammonia was measured directly in the aqueous extract, and ammonia and ammonium ions were measured after the addition of 1M sodium hydroxide, 10% by volume, to shift the ammonium ions into a molecular state. For more information of this method, see Byrne and Power (1974).

Ionic Conductivity.

The solids were oven dried and ground in a hammer mill to pass through a 2mm sieve. The material was then made up to its original moisture content and then packed into a conductivity cell with a cell constant of 1, care being taken to standardise the density in the cell. The conductivity was measured on a Digital Water Analyser in $\mu\text{S}/\text{cm}$.

2.5.5) Results.

Graph 2.5.5.1 shows the change in temperature of the composting solids under different treatments over time. Graph 2.5.5.2 shows the change in total earthworm biomass with time for the different treatments. Tables 2.5.5.1, 2 3, and 4 shows the physico-chemical attributes of the four treatments.

Table 2.5.5.1.

**Change in Variables and Earthworm Weights for
Digested solids, Hand-turned, with no Earthworms**

Day	0	14	41	63	86	119	162
total earthworm biomass (g)	-	-	-	-	-	-	-
total solids (%)	20.8	21.2	21.8	22.0	21.7	21.3	21.5
ionic ($\mu\text{S}/\text{cm}$) conductivity	5440	5390	4120	4550	4980	4730	4890
pH	9.1	9.0	7.9	7.8	8.0	7.7	7.5
$\text{NH}_3\text{-N}$ (mg/g dry wt)	0.09	0.06	0.03	0.02	0.01	<0.01	<0.01
NH_3 and $\text{NH}_4^+\text{-N}$ (mg/g dry wt)	1.63	0.72	0.32	0.14	0.05	0.07	0.03
temperature top ($^{\circ}\text{C}$)	26	25	15	17	16	17	18
temperature bottom ($^{\circ}\text{C}$)	45	28	16	17	18	17	19

Table 2.5.5.2.

**Changes in Variables and Earthworm Weights for
Digested Solids, Aerated with Earthworms Present.**

Day	0	14	41	63	86	119	162
total earthworm biomass (g)	4520	4400	3160	3000	4520	1920	1680
total solids (%)	22.8	22.2	19.2	19.0	19.4	19.2	19.7
ionic (μScm) conductivity	4540	6095	6330	6240	6370	5980	5750
pH	8.2	8.1	8.1	7.9	8.0	8.2	7.8
$\text{NH}_3\text{-N}$ (mg/g dry wt)	0.18	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
NH_3 and $\text{NH}_4^+\text{-N}$ (mg/g dry wt)	0.59	0.03	0.03	0.01	<0.01	<0.01	<0.01
temperature top ($^{\circ}\text{C}$)	11	22	20	23	22	20	21
temperature bottom ($^{\circ}\text{C}$)	20	27	23	24	23	22	22

Table 2.5.5.3.

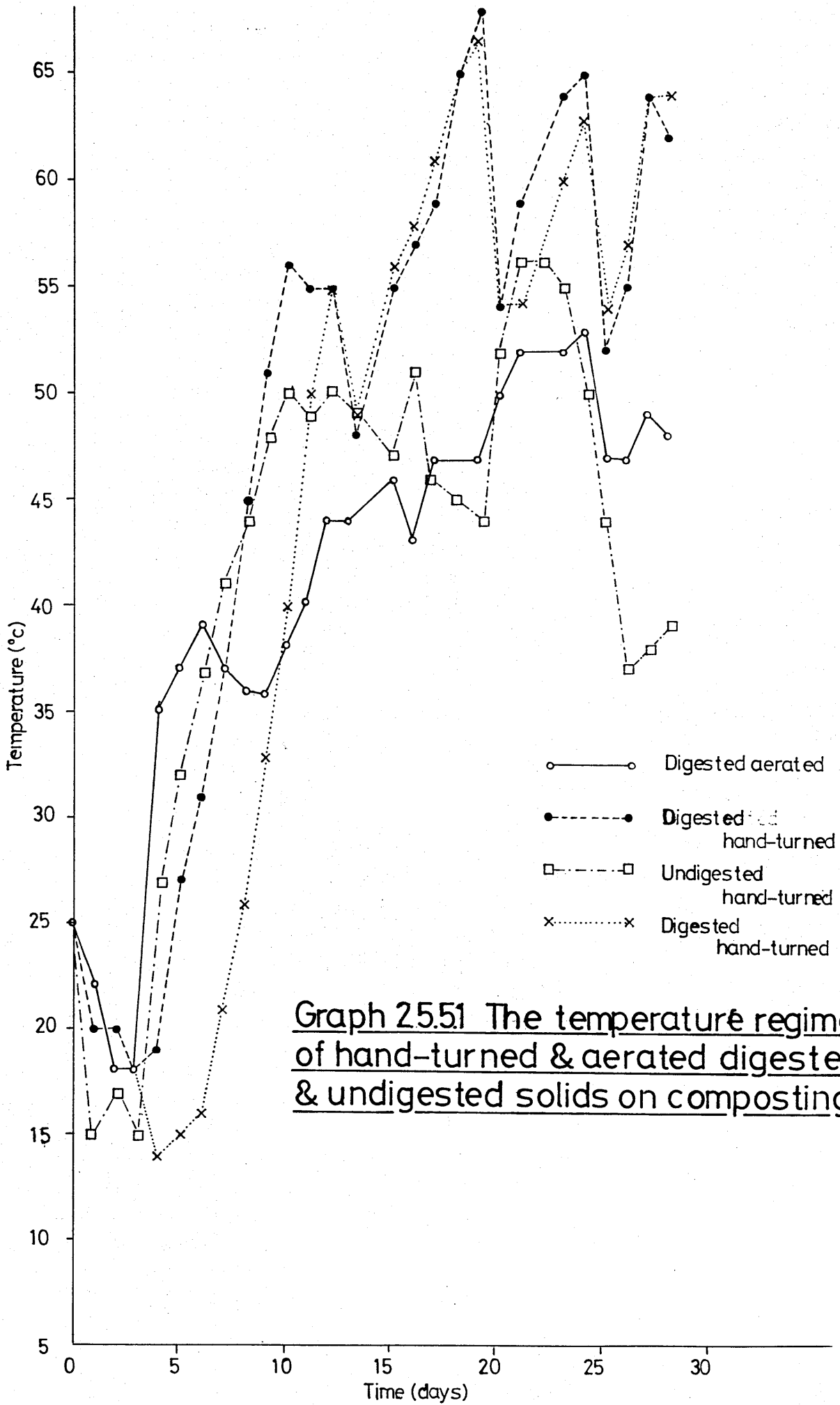
**Changes in Variables and Earthworm Weights for
Digested Solids, Hand Turned, with Earthworms.**

Day	0	14	41	63	86	119	162
total earthworm biomass (g)	4520	1320	5400	5360	4920	6520	2040
total solids (%)	22.8	20.2	20.6	20.5	20.8	21.0	21.2
ionic ($\mu\text{S}/\text{cm}$) conductivity	4460	4765	6517	5940	6130	6270	5740
pH	8.9	8.3	8.2	7.9	8.0	7.7	7.6
$\text{NH}_3\text{-N}$ (mg/g dry wt)	0.48	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
NH_3 and $\text{NH}_4^+\text{-N}$ (mg/g dry wt)	3.17	0.03	0.03	0.03	0.01	<0.01	<0.01
temperature top ($^{\circ}\text{C}$)	13	22	19	19	18	19	21
temperature bottom ($^{\circ}\text{C}$)	20	25	20	19	19	20	22

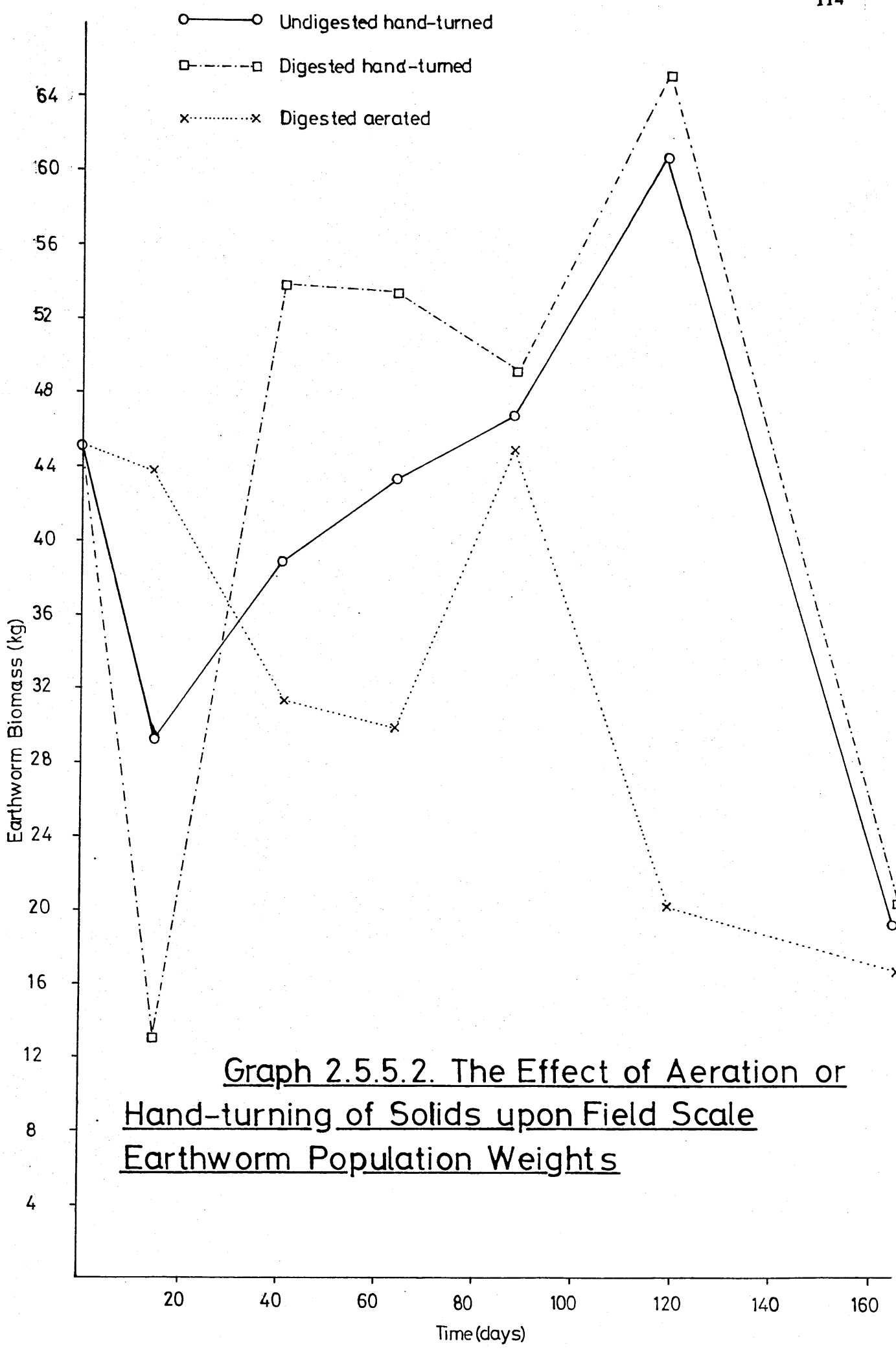
Table 2.5.5.4.

Change in Variables and Earthworm Weight for
Undigested Solids, Hand-turned, with Earthworms.

Day	0	14	41	63	86	119	162
total earthworm biomass (g)	4520	2920	3880	4360	4680	6080	1920
total solids (%)	19.9	19.5	19.1	19.3	19.7	19.9	20.5
ionic ($\mu\text{S}/\text{cm}$) conductivity	4870	5745	6833	6750	6590	6800	6450
pH	9.2	8.6	8.3	8.5	8.2	8.2	8.4
$\text{NH}_3\text{-N}$ (mg/g dry wt)	0.07	0.02	<0.01	<0.01	<0.01	<0.01	<0.01
NH_3 and $\text{NH}_4^+\text{-N}$ (mg/g dry wt)	0.77	0.13	0.02	0.01	<0.01	<0.01	<0.01
temperature top ($^{\circ}\text{C}$)	10	16	19	20	21	21	20
temperature bottom ($^{\circ}\text{C}$)	13	16	19	20	22	23	21



Graph 25.51 The temperature regime of hand-turned & aerated digested & undigested solids on composting



Graph 2.5.5.2. The Effect of Aeration or Hand-turning of Solids upon Field Scale Earthworm Population Weights

2.5.6) Discussion of Results.

A good measure of the pretreatment process is the temperature reached by the solids on composting, giving an indication of aerobic microbial metabolic processes occurring within the solids. Using this criterion graph 2.5.5.1 shows that hand-turning is a more efficient method of aerating and stimulating metabolic processes within the solid than forced aeration. This is likely to be due to the mixing and turning component of hand-turning that is absent in static pile forced aeration. In the latter, once air passages within the solids have been established, air pumped through the pile will tend to follow that route each time, with the possibility of large amounts of the material becoming or remaining anaerobic during the course of the pre-treatment process.

Similarly undigested solids do not reach the same temperature peaks as digested solids when both are hand-turned. The differences in structure and texture of undigested solids noted in section 2.2.2 also holds in this experiment, indicating that the denser undigested solids had fewer pore spaces, and so less of the material was exposed to aerobic metabolic processes, producing lower temperature maxima.

One would therefore expect to find a greater degree of decomposition in digested solids which are hand-turned than both hand-turned undigested solids and digested solids in force aerated static piles.

Graph 2.5.5.2 shows a slightly higher peak for earthworms cultured in hand-turned digested solids compared with hand-turned undigested and force-aerated digested solids. Using Students t-test at the 5% level of significance, the mean weight of earthworms sampled on day 41 for hand-turned digested solids is significantly greater than for hand-turned undigested solids, and on day 119 both the mean earthworm weight for hand-turned digested and undigested solids are significantly higher than for hand-turned undigested solids. However, by day 162 there is no significant difference between any of the groups,

and the mean weight is lower than the initial weight. The overall picture is as before, very low increases above the initial inoculum weight which tend to reduce or suppress differences between the individual treatments.

Although there is little difference between the treatments by the end of the experiment, the temperatures reached on pre-treatment, combined with the earthworm biomass data indicate that forced aeration as used in this experiment is not as good as the more labour intensive hand-turning method and requires modification before it can be used successfully. The system may require a more ramified piping network beneath the solids, a pump delivering more air per unit time, or the flow of air reversed to suck air from the outside the pile, rather than blow it from the centre. Such modifications are beyond the scope of this thesis, the main criterion for using such a system in this experiment has been satisfied by the creation of differing pretreatment conditions within the pile.

The hypothesis that pre-treatment is important in the general characteristics of the material has been borne out by the differences between aeration and hand-turning. However, the idea that the pretreatment method used is responsible for the low earthworm biomass increase in previous trials has been shown not to be the case, indicating that the earthworm response in Bore Place solids is due to some fundamental aspect of the cattle waste produced, from the physiology or diet of the cattle or slurry treatment, which cannot be investigated under field scale conditions, but require a much more detailed investigation with reference to wastes from other sources. Digested solids were shown to support earthworm growth as well as undigested solids under the prevailing experimental conditions, which, along with the results obtained in section 2.2 provides justification in continuing to evaluate its merits as a substrate for earthworm growth.

2.6) Conclusions of Field Scale Earthworm Growth Trials at Bore Place.

The initial phase of research at Bore Place has highlighted some of the many problems involved in large scale earthworm culture. These include the depth to which an earthworm bed can be filled with material, problems of high temperature and waterlogging, the implications of the stocking density used, and an initial consideration of the different methods of pretreatment. However, the most important factor has been the value of Bore Place separated cattle solids as a substrate for earthworm growth when used in field scale conditions.

The results obtained do not compare with earthworm growth data from the literature for similar animal wastes (Edwards *et al* 1985), but for the experiments in chapter two there are two variables involved, the source of the material (and therefore the characteristics of the waste pertaining to that source) and the earthworm management techniques of field scale earthworm culture, which are different to those utilised in the laboratory experiments quoted in the literature. Although the results tentatively suggest that low earthworm biomass increases are caused by a waste source related factor, the possibility of inefficient earthworm bed management cannot be ruled out.

The second important result to emerge from the initial field scale trials is that contrary to results in the literature where digested sewage sludge was found to be unsuitable for earthworm culture (Mitchell *et al* 1977), digested solids have produced generally similar or better results than undigested solids in the experimental conditions described, warranting further research into their value as a substrate for earthworm culture.

In order to elucidate more fully the problem of linking waste source characteristics or earthworm management techniques to the earthworm growth data obtained, and to better assess digested solids, further field scale growth trials are proposed, keeping the experimental design as simple as possible to reduce problems of bed management and to obtain

comparisons of different types of waste from Bore Place.

Chapter Three

Undigested and Digested Solids as Substrates for Field Scale Earthworm Culture.

3.1) Introduction.

The earthworm growth results from chapter two show lower earthworm growth rates than expected from the published data. Likely causes have been identified as a factor relating to the source of the separated solids, or some aspect of earthworm bed management which is inhibiting earthworm growth. The experimental framework can be improved by simplifying the experimental plan, improving the earthworm bed design and using the knowledge gained in earlier experiments. Comparisons of three different types of separated solids from Bore Place, combined with physico-chemical analyses will allow an investigation of the hypotheses that low biomass increases are due to factors within the solids arising from the physiology or diet of the cattle, or treatment of the slurry prior to separation. The construction of an outdoor earthworm culture facility at the Open University allowed experiments of a similar scale to those at Bore Place be carried out, but with access to laboratory facilities.

There were some fundamental flaws in the design of the earthworm beds at Bore Place. The 1m height of the walls was greater than necessary to contain the solids, as was subsequently shown in section 2.3, where bed depths above 50cm caused temperature increases that would inhibit earthworm growth. The height of the walls made access to the beds difficult and impeded experimental manipulation. More importantly the drainage system of the beds did not function as efficiently as expected. This was mainly due to the 'teram' material used to line the beds, through which water passed at a very low rate. No drainage water was ever seen issuing from the concrete channels. Given the high moisture holding capacity of separated solids any barrier to drainage will tend to cause water to remain in place. Such factors may in part account for the poor earthworm growth observed in earlier sections.

The experimental plan in chapter three is to observe the growth and reproduction of a fixed inoculum of earthworms in a fixed amount of undigested and digested solids and

farmyard manure (FYM), as measured by regular sampling. By having an experimental design without complication, and by carrying out physico-chemical analyses of the solids it is hoped that the underlying earthworm growth trends observed in earlier experiments can be better understood.

3.2) Description of Open University Earthworm Tunnel.

The tunnel was designed to give easy access to the maximum number of approximately 1m^2 beds within the confines of a polyethylene tunnel 4m by 12m. The beds are of brick construction with wooden boarding at the front for easy loading and unloading of cattle solids into the beds. The base of the beds is on a layer of sand and gravel to improve drainage. Figures 3.2.1 and 2 and photograph 3.2.1 show the layout of the beds, six on each side, with an area of 0.69 m^2 per bed. An undersoil heating cable was installed at the bottom of the beds in order to control temperature within solids placed in the beds.

Figure 3.21. Layout of Earthworm beds

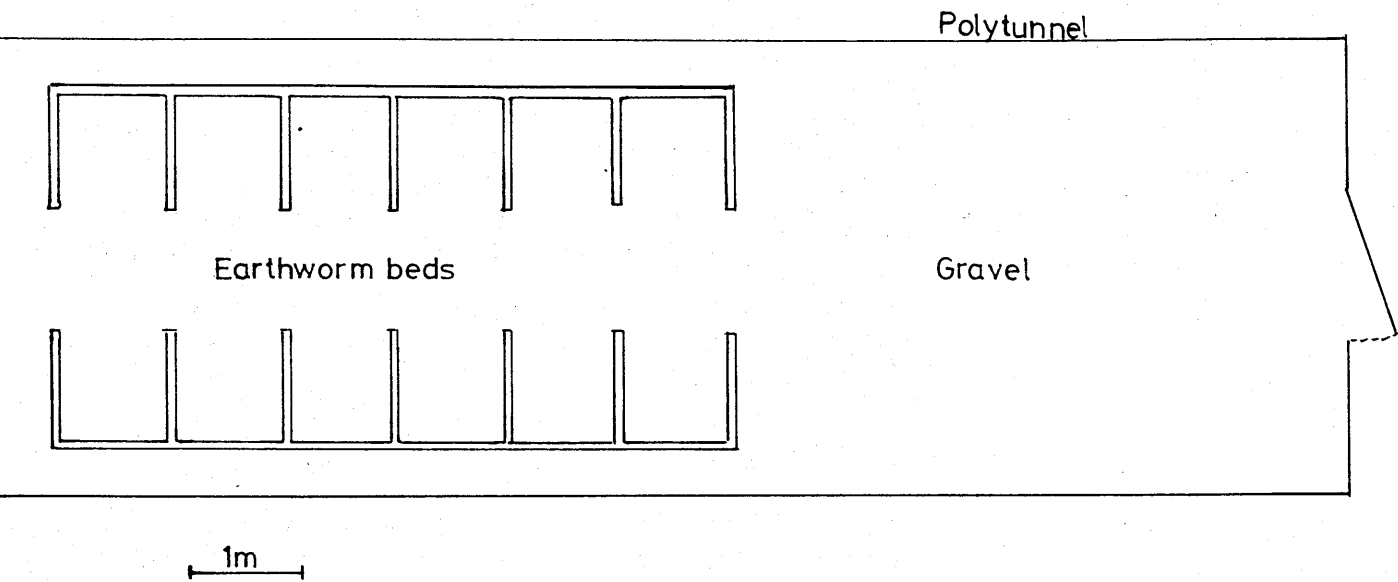
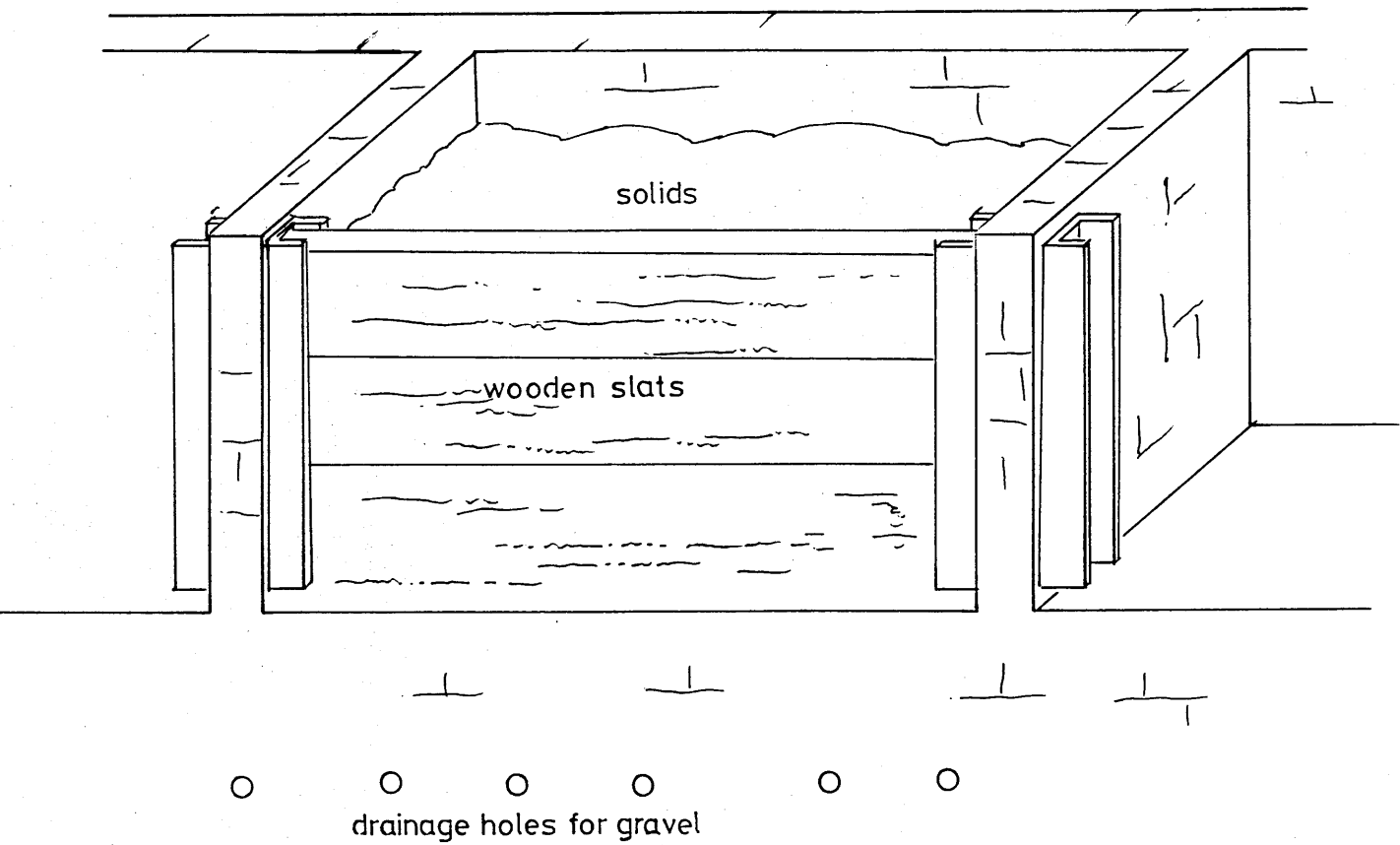
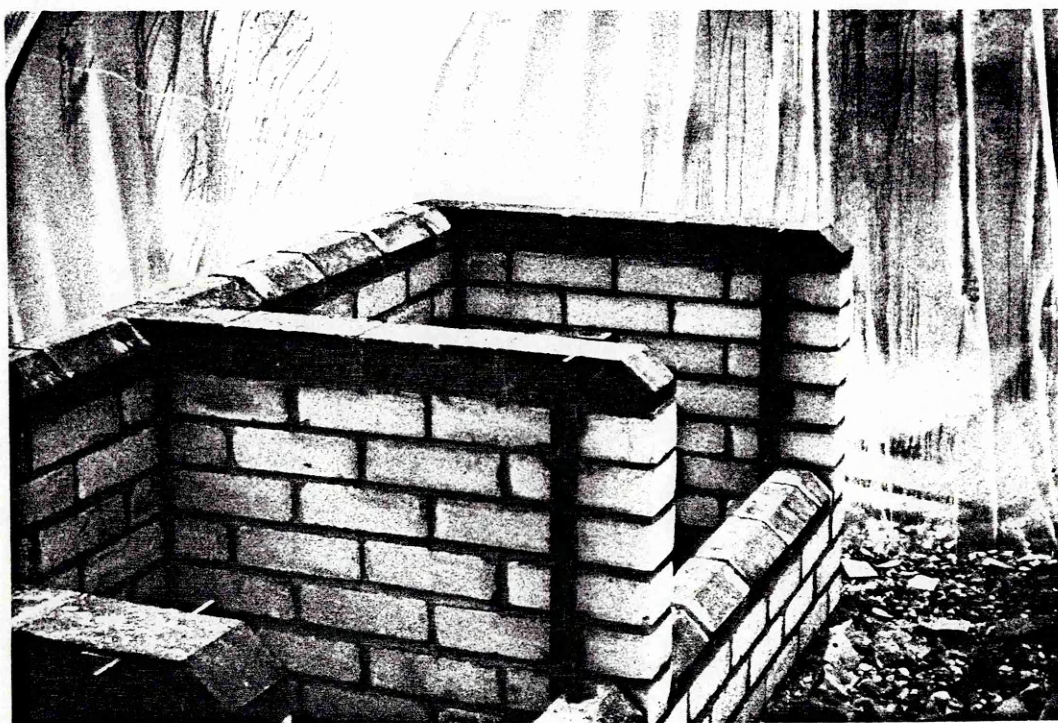


Figure 3.2.2. An Individual Earthworm bed



Photograph 3.2.1.

One of Six Earthworm Beds on Each Side of a
Clear Polytunnel at the Open University.



3.3) Experimental Design.

The purpose of the experiment was to monitor earthworm growth in three types of cattle solid from Bore Place under simple experimental conditions, and to analyse some physico-chemical factors within the solid as it undergoes physico-chemical changes to become acceptable to earthworms. In order to follow these changes the solids were loaded directly into the relevant beds in their fresh states and 'artificially' composted by heating the solids within the beds via an undersoil heating cable. This gives a much greater degree of control over the process compared to natural composting of solids in a large midden or heap, as temperature is a good indicator of the progress of the composting process.

Inoculation with earthworms took place once the solids had become acceptable, and the beds then regularly sampled over 120 days to monitor the earthworm population. Over this period no additional treatments were carried out, so keeping the experimental design as basic as possible.

3.4) Experimental Procedure.

The separated solids and FYM used in this experiment were collected from Bore Place and transported to the Open University in their fresh state, and loaded into the earthworm beds in the following manner;

Bed 1 Undigested Solids	Bed 7 Undigested Solids
Bed 2 FYM	Bed 8 Digested Solids
Bed 3 FYM	Bed 9 Digested Solids
Bed 4 FYM	Bed 10 Digested Solids
Bed 5 FYM	Bed 11 Digested Solids
Bed 6 Undigested Solids	Bed 12 Undigested Solids

Although laid out in this manner for convenience, in retrospect the non-random nature of the distribution of the solids represents a potential source of between-treatment differences generated by the position of the treatment rather than the type of material assigned to it. This is discussed more fully in section 3.9, where it is argued that any positioning effects on the results are minimal, but if time were available the experiment should have been repeated adopting completely random bed positions for the three treatments.

Approximately 100kg fresh weight FYM was loaded into each of beds 2, 3, 4 and 5. 200kg fresh weight of undigested solids was loaded into beds 1, 6, 7 and 12, and 200kg fresh weight digested solids was loaded into beds 8, 9, 10 and 11.

The heating cable was left on for a period of three weeks in order to raise the temperature in the beds to initiate the thermophilic processes of composting. The beds were turned manually every week to reduce anaerobiosis in the lower levels of the beds. Random samples were taken regularly from each of the beds for physico-chemical analysis (see section 3.3.4).

3.5) Inoculation and Sampling of Earthworm Beds.

The earthworm beds were inoculated 52 days after being loaded with fresh solids. A total of 172g live weight *E.fetida* were placed in three of the four beds containing undigested or digested solids or FYM. The fourth bed remained free of earthworms as a control.

The size class distribution of the *E.fetida* population used as inoculum can be seen in graph 3.3.5.1. Of note is the very high numbers of hatchlings in the inoculum. The earthworms and a small amount of associated solids were put in the base of each bed, and the solids then placed on top.

Sampling was carried out on five occasions, 10, 26, 54, 81, and 118 days after inoculation. In each case a plastic corer of internal diameter 10.3cm was used to take three samples at random from the beds. The earthworms and cocoons were hand sorted from the solids, and each earthworm washed, dried on a paper towel, weighed and placed in 0.05g size classes.

The results from the three cores were combined to produce an estimate of the earthworm population in the bed. The results for each of the three beds inoculated were combined to produce a mean result for each of the three types of solid used.

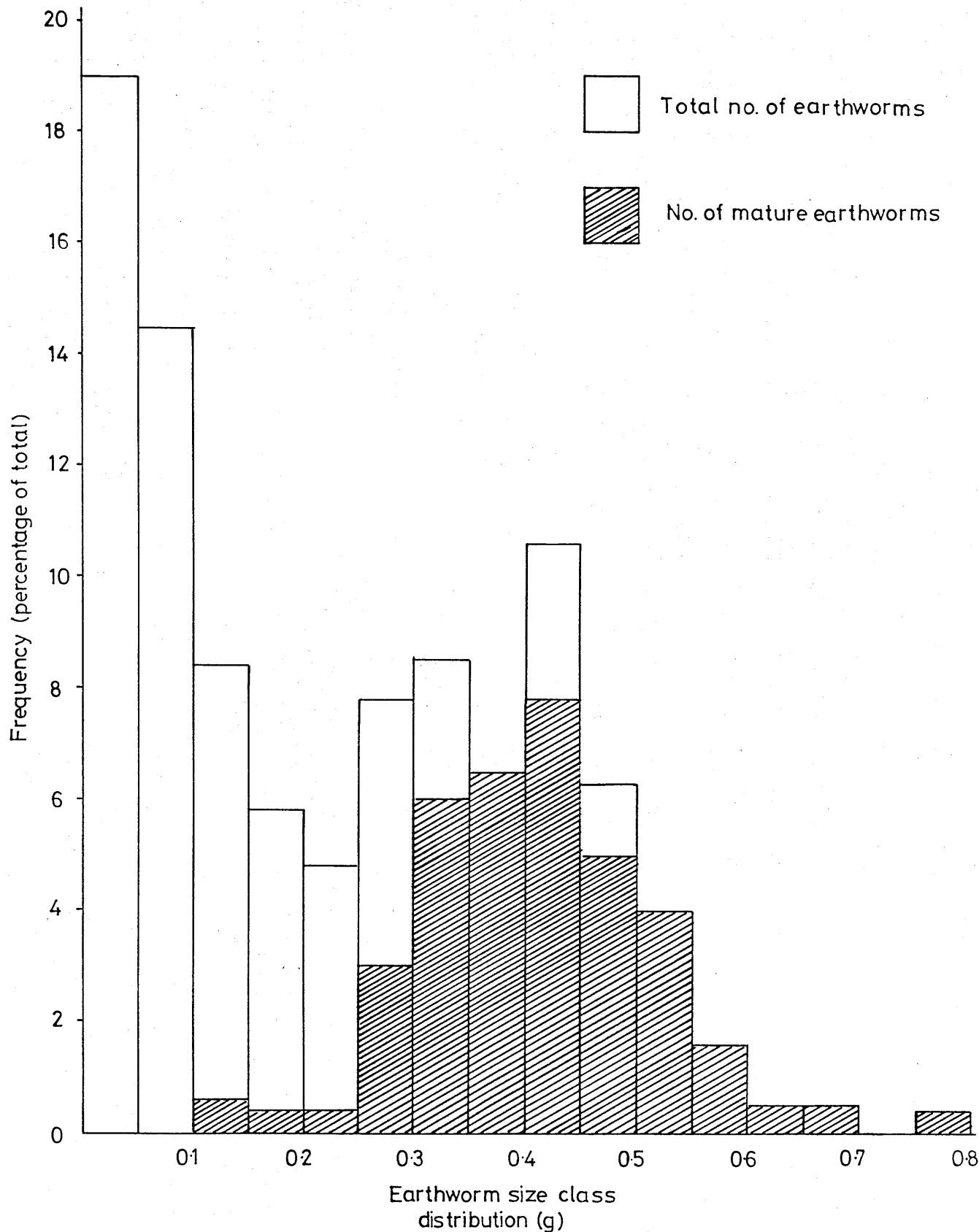
3.6) Physico-chemical analyses of composting solids.

The solids were sampled 0, ~~7~~¹⁰, 14, 21, 29, 44, and 52 days after loading the beds with fresh material, and analysed as in section 2.5.4.

3.7) Results.

Table 3.7.1 shows the results of physico-chemical analyses for digested solids, undigested solids and FYM respectively. Graph 3.7.1 shows the size class distribution of the inoculum used in the experiment and graph 3.7.2. shows the change in mean earthworm biomass for each solid over time.

Graph 3.7.1. Weight Distribution of Earthworm Inoculum



Graph 3.7.2. Changes in Earthworm Population Biomass in Digested and Undigested Solids

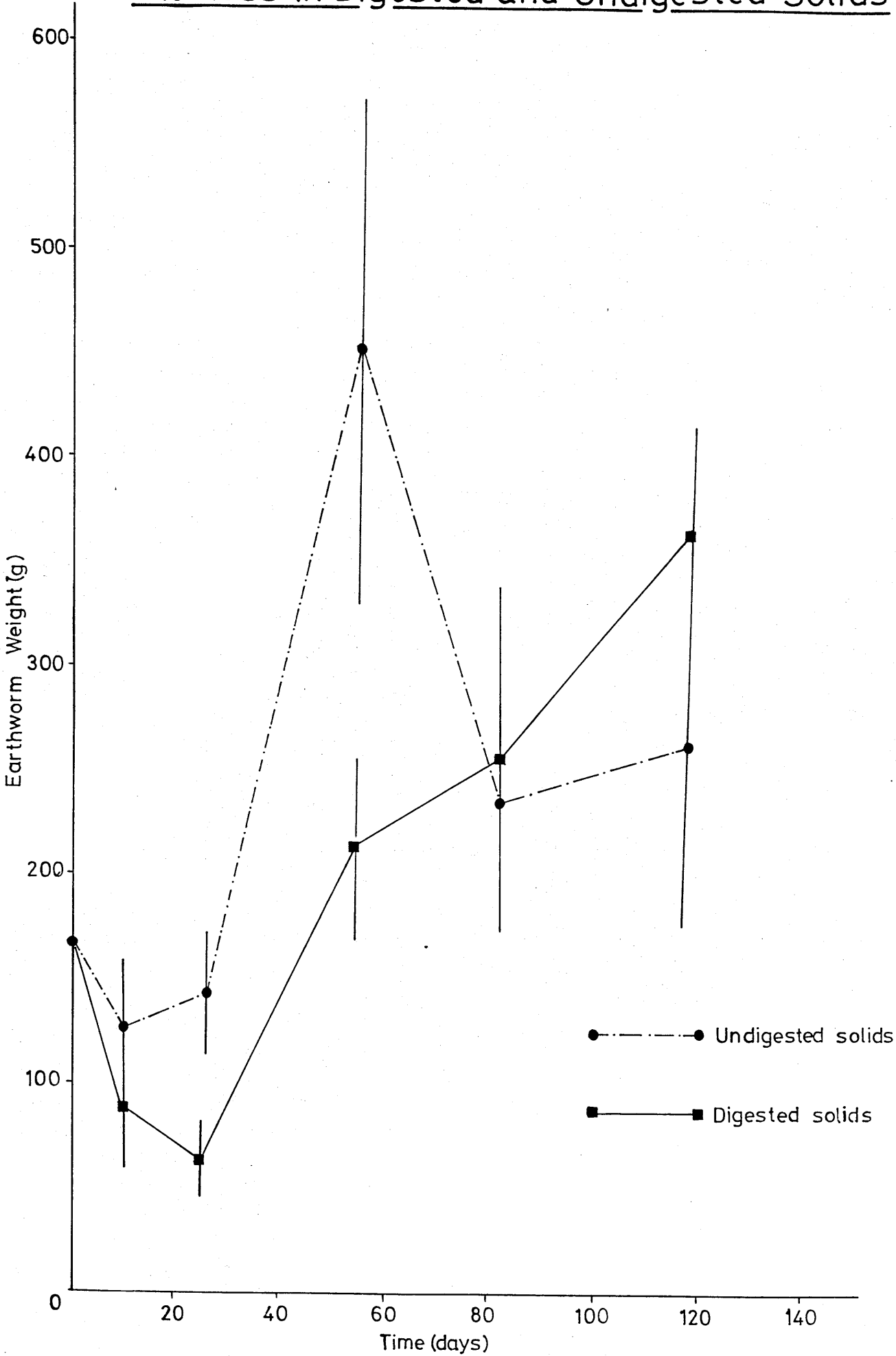


Table 3.7.1.

**Changes in Physico-Chemical Characteristics on Composting
of Undigested and Digested Solids and Farmyard Manure.**

Variable	Type of Solid	Day 0	Day 7	Day 14	Day 21	Day 29	Day 44	Day 52
Temp (°c)	DS	9	49	60	57	27	25	20
	US	11	48	52	47	21	19	17
	FYM	9	40	47	45	21	19	18
T.S. (%)	DS	21.2	21.2	21.9	23.2	23.9	24.2	23.0
	US	20.1	20.4	20.1	20.1	21.5	21.7	21.3
	FYM	19.1	20.0	18.6	20.5	18.3	17.7	18.1
pH	DS	8.5	8.6	8.5	8.3	8.2	8.4	7.9
	US	8.3	8.3	8.2	8.3	8.1	7.9	8.2
	FYM	8.2	8.5	8.1	8.1	7.9	7.6	7.7
Ionic C. (μ S/cm)	DS	4860	2350	2750	2980	3250	3790	4260
	US	4320	3200	3350	3690	4140	3920	4750
	FYM	6020	5900	5740	5770	5320	4970	5110
NH ₃ -N (mg/g dry)	DS	0.78	1.22	0.81	0.28	<0.01	<0.01	<0.01
	US	0.24	0.26	0.13	0.02	<0.01	<0.01	<0.01
	FYM	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
NH ₃ -N and NH ₄ ⁺ -N (mg/g dry)	DS	7.23	7.32	4.60	3.11	0.08	1.30	1.15
	US	5.56	3.05	0.89	0.09	0.02	0.02	0.01
	FYM	0.04	0.02	0.02	0.04	0.02	0.01	0.01
Bulk Density (kg/m ³)	DS	590	620	690	750	770	780	800
	US	850	920	980	1030	1050	1060	1060
	FYM	520	570	650	640	660	640	670

DS = Digested Solids

US = Undigested Solids

FYM = Farmyard Manure

3.8) Discussion of Results of Physico-Chemical Analyses.

Comparison between digested and undigested solids.

The results show that digested solids reached a higher temperature during the composting process than did undigested solids. As the initial increase in temperature came about through undersoil heating cables the higher temperatures recorded in digested material may therefore correspond to physical attributes of the solid to a greater degree than any biochemical differences influencing the rate of microbial metabolic processes. The lower bulk density of digested solids means that it has a higher proportion of air spaces, and so better insulating properties, and this could account for the observed differences.

Digested solids also have a lower moisture content than undigested solids. This is probably a consequence of the differing moisture contents and particle size distribution of the two slurries. Input slurry moisture content has a direct effect on separated solids moisture content during the process of mechanical separation (Pain *et al* 1978). This too may allow better heat retention within digested solids, as air in pore spaces will provide better insulation than water.

There are higher concentrations of ammonia and ammonium ions in digested solids. Theoretically there is no loss of nitrogen during anaerobic digestion, only methane, water, and CO₂ and some sulphur compounds in low concentrations. Hobson (1985) states that there is very little change in the form of N during digestion, although Hill (1980) noted an increase in NH₃-N from 657.5 mg/l in influent slurry to 2042.4 mg/l in effluent slurry for dairy manure of high solids concentration. This apparent anomaly can be explained if very rapid changes occur in the period directly after digester effluent discharge. A large obligate anaerobic microflora will develop during the process of digestion which will be exposed to air on discharge, changing the microhabitat and causing microbial death and decay. On discharge one could therefore expect a rapid conversion of microbial N to

$\text{NH}_3\text{-N}$, some of which will pass into the solid fraction with associated liquid, leading to higher NH_3 and NH_4^+ ions in digested solids.

The dissociation of free ammonia to an ionic form causes a release of hydroxide ions. This will raise the pH and may be responsible for the consistently higher pH levels recorded in digested solids.

The reduction of $\text{NH}_3\text{-N}$ and $\text{NH}_4^+\text{-N}$ concentrations over time may have occurred through three processes, volatilisation, nitrification and microbial immobilisation. High pH and temperature enhances NH_3 volatilisation (Freney *et al* 1983) and high NH_3 concentration inhibits nitrification (Sharma and Ahlert 1977) indicating the importance of the former over the latter, with an undefined proportion of NH_3 or NH_4^+ ions being taken up by the microbial biomass.

The ionic conductivity values follow a similar pattern in digested and undigested solids, an initial drop in conductivity followed by a slow rise. The loss of NH_4^+ ions and microbial uptake will account for some of the decrease, and the subsequent increase may be apparent as a result of the loss of moisture from the solids, or the loss of volatile solids that will have occurred, although not measured.

Physico-chemical analysis of FYM.

The FYM used in this experiment was found to have a very low faecal material/straw ratio, and so an unsatisfactory C/N ratio for organic decomposition, as indicated by the $\text{NH}_3\text{-N}$ and $\text{NH}_4^+\text{-N}$ concentrations. There was little evidence of straw decomposition over the 52 day composting period.

3.9) Discussion of Earthworm Sampling Results.

The most obvious feature from graph 3.7.2 is the large peak in earthworm weight in undigested solids, with an equally large drop in weight afterwards. This is a very unusual result, and only a dramatic change in the conditions of the bed, causing many earthworm mortalities, could explain the fall in earthworm population. It is not clear that such a catastrophic event occurred and the other possibility is that the samples taken were unrepresentative of the actual population present. This is covered in section 3.10.

The mean results of earthworm weight in digested solids follows a more familiar pattern, as observed in the final Bore Place experiment (section 2.5), of an initial drop in the total earthworm weight followed by a very slow increase over the course of the experiment. However for both undigested and digested solids, the increases over a small initial inoculum (172g live weight of earthworms) are not great. Considering that a very large number of hatchlings were present in the original inoculum, one might expect large weight increases in the first six or seven weeks as the hatchlings grew to sexual maturity. The initial drop in weight from the inoculum therefore indicates non-ideal conditions. Very low NH_3 and NH_4^+ ion concentrations were recorded at the end of the 52 day composting period, within limits of earthworm tolerance described in section 1.5.5.

The final ionic conductivity values recorded at the end of the composting period for digested and undigested solids were 4260 and 4750 $\mu\text{S}/\text{cm}$ respectively. A figure of 7000 $\mu\text{S}/\text{cm}$ was quoted by Edwards *et al* (1985) as the upper conductivity limit tolerated by earthworms, and this is supported by the work of Bryson (1984). However, the ionic conductivity value may still be producing an inhibition effect in one of two ways. Hartenstein *et al* (1981) showed that certain salts produced a significant reduction in earthworm weight at non-lethal conductivity levels, and so it is possible that the presence of a particular salt in digested and undigested solids inhibited earthworm growth. Secondly, there has been no comprehensive study on the general effects of non-lethal conductivity

levels on earthworm growth, so that as well as specific salts causing inhibition, high conductivities as found in this experiment may generally inhibit growth. There is scope for further research here.

Another possible explanation for the initial drop in earthworm numbers was a rise in temperature immediately after inoculation, peaking after day 8 for undigested solids and day 9 for digested solids, with temperatures of 31 and 33.5°C respectively. The cause of the temperature increase in the solids was an increase in air temperature to a maximum of between 35-45°C from 1st June to 8th July because of a period of good weather and continuous sunshine. This raised the temperature in the beds, despite opening all doors and installing electric fans to increase air movement. The 2.5°C temperature difference between undigested and digested solids may be accounted for by the exposed outer wall of the beds that ~~undigested~~ solids were placed in, and would therefore represent an introduced error arising from the non-random distribution of the treatments. However, a brick wall has fairly good insulation properties, and represents only a small proportion of the surface area of the material from which heat could be lost. A more likely cause of the difference is the intrinsic insulating properties of the two types of separated solids themselves, influenced by their respective moisture contents and particle size.

It is difficult to know if these elevated temperatures were responsible for any earthworm mortalities. Hartenstein (1982) found that 100% of earthworms in an experimental culture survived after 30 days at a temperature of 30°C, except at a density of 8 earthworms per 30g of sludge, where there was only a 80% survival rate. In contrast, Grant (1955) found that 55% of *E. fetida* died after 6 hours exposure to temperatures of 30°C in chambers containing saturated air and immersed in a water bath. The test earthworms had previously been adapted to 22°C. At 33°C Kaplan *et al* (1980) found a 70% mortality in experimental populations, with the remainder losing weight.

Such data would suggest that the temperatures observed in the earthworm beds were high

enough to cause at least some mortalities over the 3 to 4 day period that elevated temperatures were recorded. It is possible that the susceptibility of hatchlings to increased temperature would be greater than that of earthworms with a larger body weight, in which case the rise in temperature would have an even bigger effect given the large numbers of hatchlings in the original inoculum. However, to set against this is the ability of earthworms to sense temperature and move along temperature gradients, as described by Grant (1955).

The recorded temperature of the beds was measured with a mercury thermometer placed in the centre of the beds to a depth of 15cm, giving the maximum value. The periphery was at least 5°C cooler, allowing earthworms to move away from high temperatures. Such a dispersion would make sampling difficult in assessing a true value of earthworm numbers and biomass, because of non-random distribution of earthworms within the bulk of the solids.

It is possible therefore that the large peak in earthworm numbers in undigested solids could result from a core sampling a chance cluster or congregation of earthworms.

Farmyard manure.

After 26 days the earthworm population within all three beds had dwindled to a residual number which was too small to be recorded by core sampling.

Such results could be expected because of the very low nitrogen levels within the FYM leading to high C/N ratios and very little decomposition of the carbonaceous straw. In the 52 days from the loading of the beds to the introduction of the inoculum only a small amount of the cellulose within the straw would have broken down, offering very little nutrition for the earthworm. A visual inspection of the beds showed no breakdown of the whole straw lengths, and earthworm activity was confined to areas where cattle

faeces were concentrated.

Although this is an extreme case, it indicates the problems that are encountered in dealing with straw based wastes. A high N source is required to initiate the decomposition of cellulose and hemi-cellulose, and the large particle size of the straw means that if there is no initial decomposition of the straw then the earthworms cannot physically ingest the material and so accelerate the rate of decomposition.

3.10) Problems Associated with Field Scale Trials.

In a field population with many hundreds or thousands of earthworms of different weights and stages of sexual development, and with many cocoons, one is dealing with a very dynamic situation, changing in both time and space as earthworms grow, reproduce and die. The solids which they inhabit are not static, but change in physical and chemical characteristics, both vertically and horizontally, and also in time through independent microbial processes, as well as earthworm induced changes.

The only way to follow earthworm population and physico-chemical change is through sampling. The best sample to take is the entire earthworm bed in question, but this is not feasible, and so sub-samples must be taken, and the more taken the more accurate the extrapolated result.

The criteria for choosing sample numbers taken in previous experiments was a compromise between the time available to process the samples and the minimum requirement for statistical analysis. The low earthworm numbers obtained in most of the field trials utilising solids derived from Bore Place adds to the problem, as the low densities of earthworms obtained made representative sampling more difficult. This was especially the case if conditions within the solid were not homogenous, causing a non-random distribution of earthworms. Two such cases encountered were anaerobic conditions developing in the base of poorly drained beds, and the temperature variation that can develop between the centre and the periphery of an earthworm bed.

Overall, non-optimum conditions which lead to earthworm clustering and generally low earthworm populations require more samples to record accurately than large active earthworm populations. The question arises as to whether very accurate figures for low populations actually increases the amount of information that can be obtained from the experiment.

3.11) General Conclusions of Field Scale Earthworm Trials.

The common theme throughout these initial field scale trials has been the low increases or even decreases in total earthworm biomass in all of the experimental treatments.

Although some particular factors have been shown to be important in inhibiting earthworm biomass increase, such as temperature, anaerobic conditions, high C/N ratios and the form of solid pretreatment, the results suggest that such factors are reducing already low earthworm biomasses. This strongly suggests some overall factor which is having an inhibiting effect upon every experimental, and is acting independently of the factors mentioned above. The two most likely candidates are the adoption of some incorrect earthworm culture management technique, or a factor related to the source of the separated cattle solids. There is also the possibility that the earthworms used as the inocula were inferior in some way, and so did not grow and reproduce in a manner representative of the species. However in the course of the four field scale trials earthworms from several different sources were used, and it is unlikely that they were all obtained from inferior stock.

The experiments in chapter three sought to minimise the need for the management of the beds by streamlining the experimental procedure in light of experience gained from earlier experiments, and still only very low earthworm biomass increases were recorded. This leads to the conclusion that the source of the separated solids, affecting both digested and undigested solids is influencing its ability to support earthworm growth, stemming from the diet of the dairy cattle, their physiology, or the manner in which the slurry is handled prior to separation or digestion and separation, producing particular physico-chemical characteristics which are not conducive to earthworm growth.

One can state that separated solids from Bore Place do not provide a good substrate for earthworm growth. Why it is 'not very good', and how it can be characterised in terms

of physico-chemical factors and wastes from other sources is the objective of further study. However, further field scale trials are not a useful method for gaining new information in this area. A more closely defined system is required whereby the variables involved can be controlled more easily. This can be achieved through the use of laboratory scale experiments which allow a greater degree of experimental control over the earthworms and the organic waste.

Chapter four

Initial Laboratory Studies on the Growth and Reproduction of *E.fetida* in Cattle Solids.

4.1) Introduction.

Field scale trials have shown very little increase in earthworm population size over the original inoculum, with no obvious causes. There is no doubt that large earthworm populations can be supported by organic wastes (Edwards *et al* 1985) and the theoretical population increase from life history and reproductive data (section 1.4.3) is far in excess of the observed results, given the time over which the experiments ran. Possible reasons for this have been discussed in section 3.5.

In order to examine this more fully the system needs to be studied in much greater detail than is possible on a large scale situation, indicating the need for carefully controlled laboratory experiments, where the physico-chemical characteristics of cattle solids outlined in earlier sections can be measured and manipulated. Also, this inhibition effect noted in field scale trials has also tended to obscure any differences between digested and undigested solids from Bore Place, and although the results obtained to date indicate that digested solids can be used as a substrate for earthworm growth, more definitive results are needed to substantiate this, which can be contained from laboratory scale comparisons between the two materials, which at the same time produce data on the capacity of waste from Bore Place to support earthworm growth and reproduction.

4.2) The Growth of *E.fetida* in Fixed Amounts of Undigested and Digested Cattle Solids and at different stocking densities.

4.2.1) Introduction.

In this section undigested and digested separated cattle solids from Bore Place is used for individual earthworm growth trials in the laboratory in order to obtain basic growth data which may allow a better interpretation of the preceeding work. The experimental design is deliberately uncomplicated to avoid extraneous factors. The first experiment follows the growth of individual *E.fetida* in a fixed amount of digested and undigested cattle solids. In the second experiment earthworms at four stocking densities are used.

4.2.2) Materials and Methods.

Well composted undigested and digested cattle solids were taken and analysed for moisture content. The undigested solids were found to have the highest moisture content, and 60g fresh weight was weighed out and placed in each of 20 10cm diameter plastic containers with tight fitting plastic lids, holed for ventilation. For digested solids, slightly less than 60g was placed in each container, and the difference made up with an amount of distilled water, calculated to equalise the moisture level contents for each solid.

Young hatchlings of *E.fetida* with a live weight of between 0.01 and 0.03g were hand sorted from an active earthworm population and one placed in each of the 40 containers with undigested or digested solids. Once inoculated the containers were placed in an incubator set at a constant $20 \pm 1^{\circ}\text{C}$. In order to control the temperature further the laboratory was fitted with two hot air fans, connected to a thermostat set at $20 \pm 2^{\circ}\text{C}$, controlling the ambient laboratory air temperature 24 hours per day.

The containers were removed from the incubator approximately every seven days, and

the earthworms removed from the solids with a pair of forceps, rinsed in distilled water to remove any attached solid particles, and dabbed dry on absorbant paper. When the excess moisture had been removed from the body of the earthworm it was weighed on a Sartorius balance to the nearest 0.01g and then replaced in the relevant container. As in Neuhauser *et al* (1981) no attempt was made to void the gut contents of the earthworms, because of the attendant stresses involved. the gut content may account for up to 10% of the liveweight of an earthworm. The experiment was conducted over a period of 20 weeks.

The second experiment followed the same procedure as above, except that 1, 2, 4 or 8 earthworms were placed in each container, with five replicates of each treatment.

4.2.3) Results.

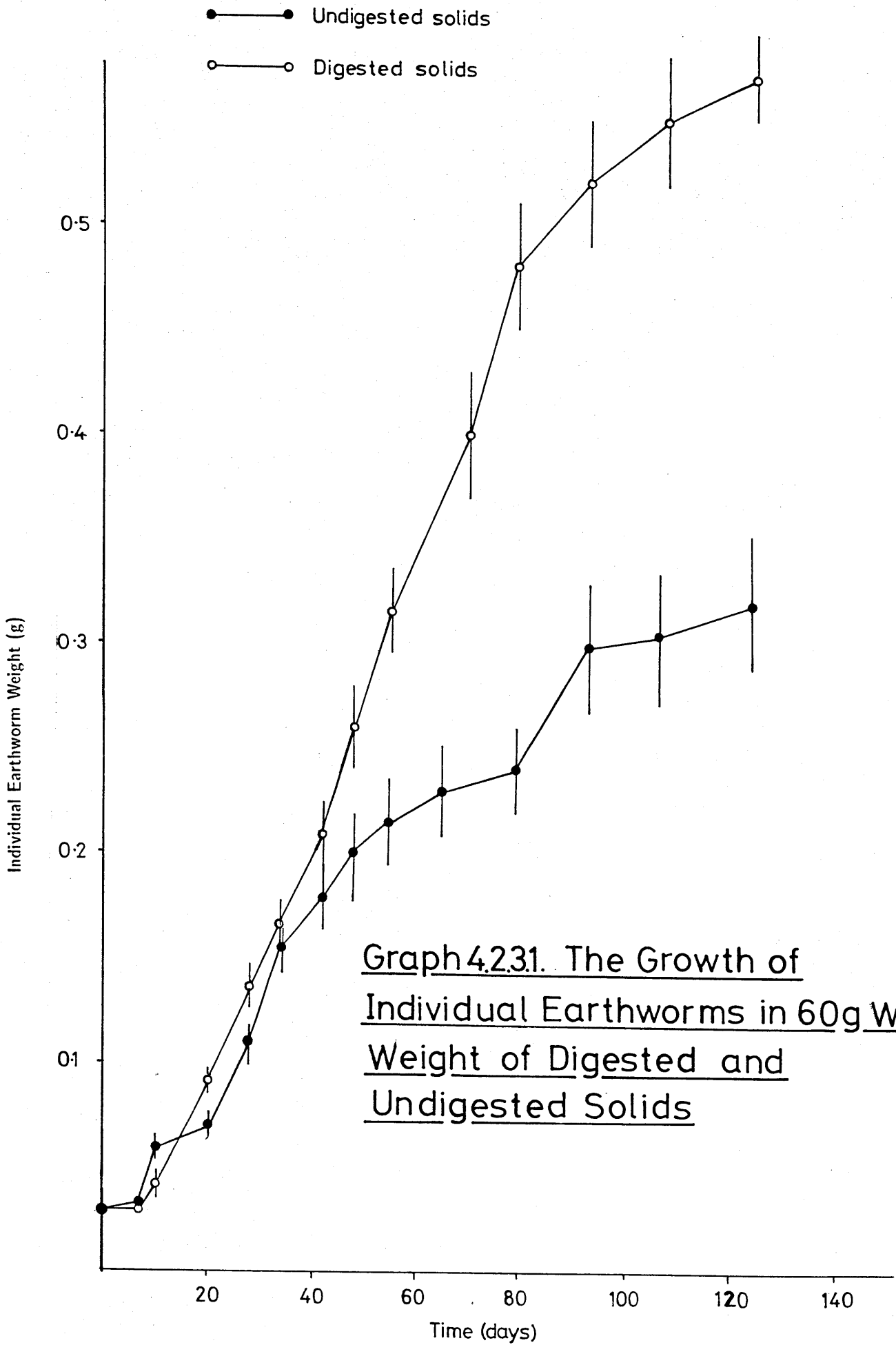
Graph 4.2.3.1 shows the change in mean earthworm weight in time, and graph 4.2.3.2. shows the change in the percentage of sexually mature earthworms over time as indicated by the presence of clitella. Table 4.2.3.1 shows the results of physico-chemical analyses of the solids. Graphs 4.2.3.3 and 4 shows the mean earthworm weight in different stocking densities for undigested and digested solids.

Table 4.2.3.1.

Analysis of Bore Place Digested and Undigested Solids.

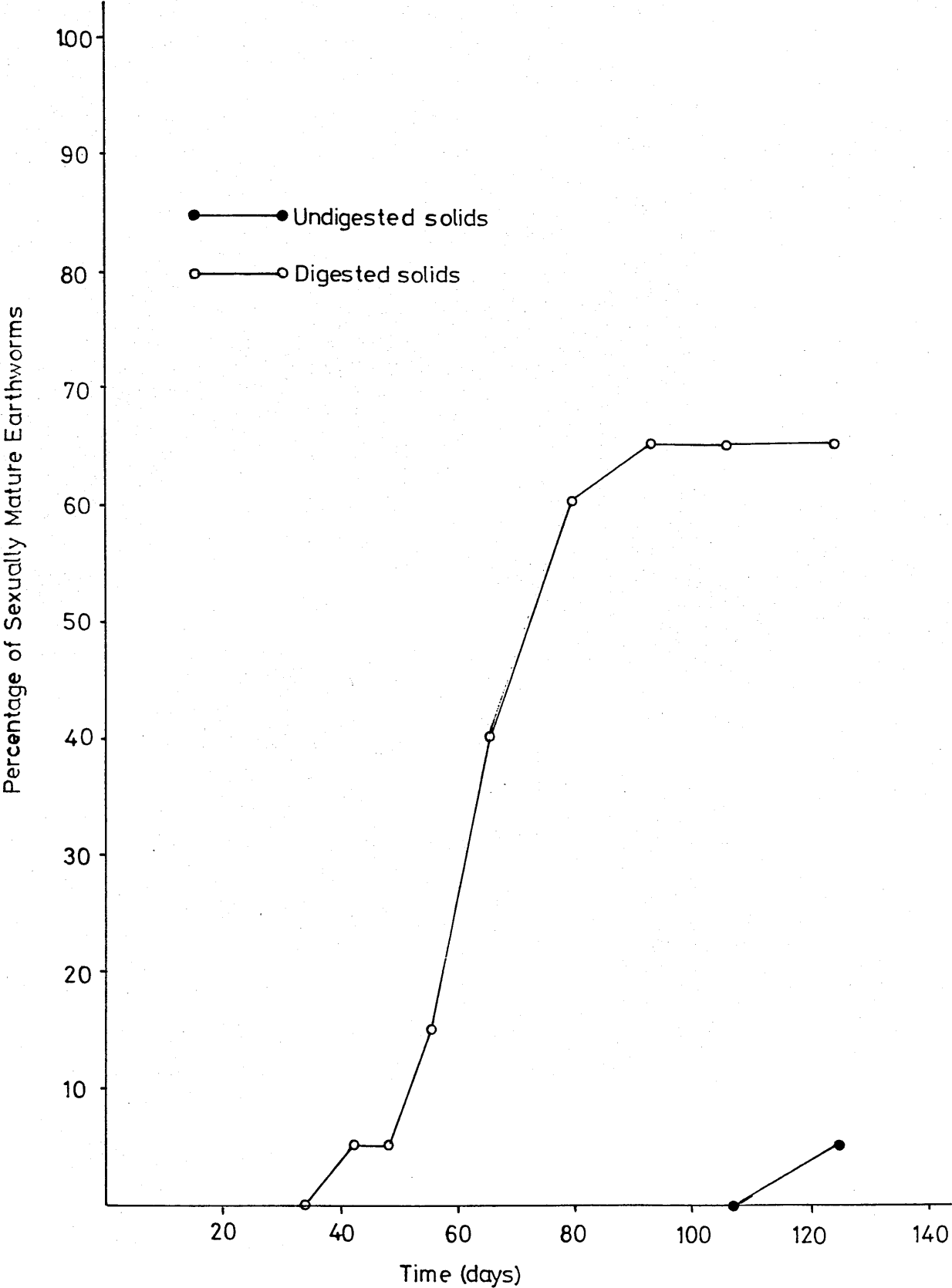
Variable #	Undigested Solids	Digested Solids
T.S (%) *	23.7%	25.3%
Initial pH	8.7	8.7
Final pH	8.8	8.0
Ionic Conductivity (μS/cm ²)	5350	4050
NH ₃ -N (mg/g dry wt)	0.01	0.02
NH ₃ and NH ₄ ⁺ -N (mg/g dry wt)	0.02	0.02

* Moisture content before experimental adjustment

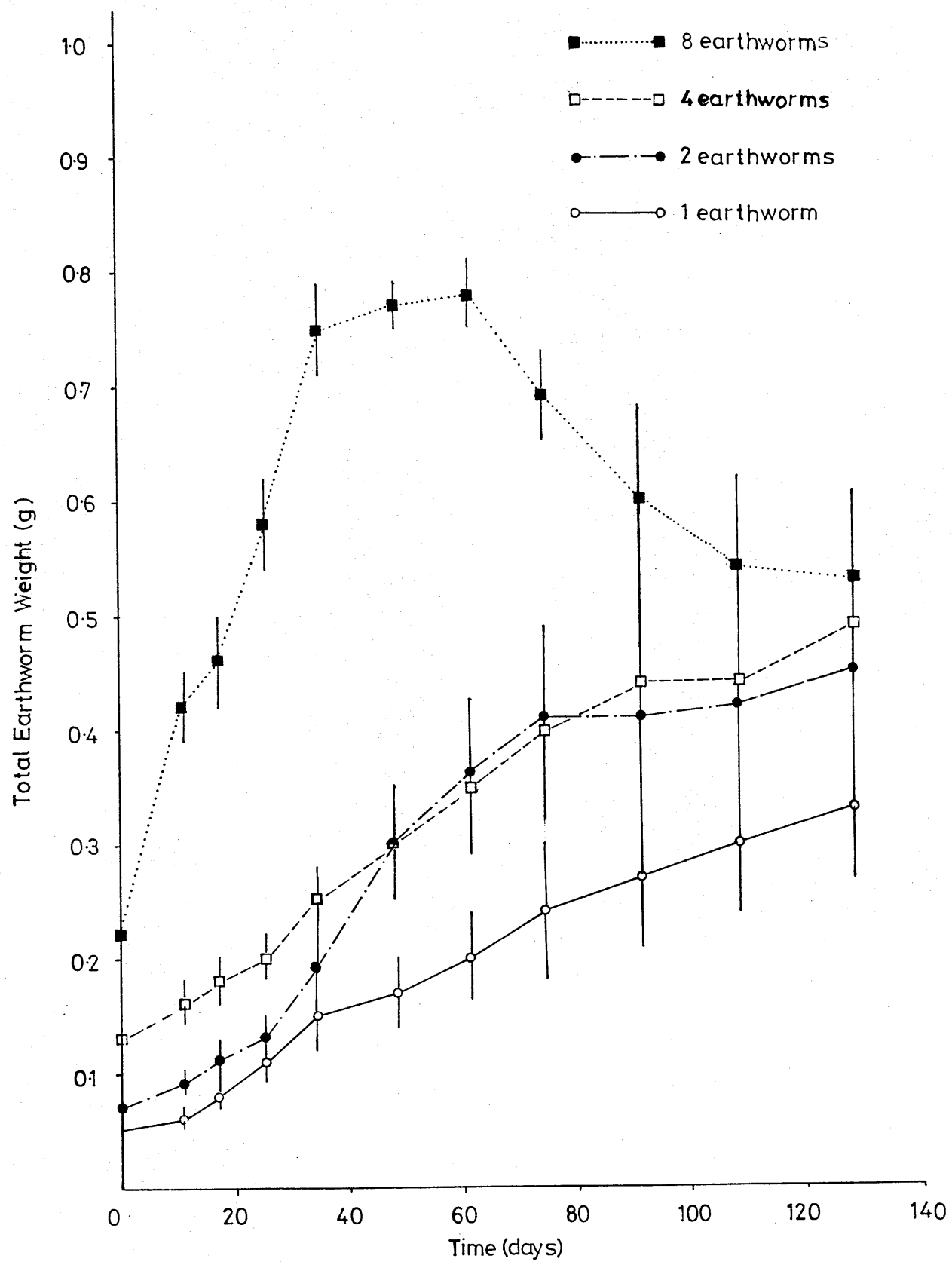


Graph 4.231. The Growth of Individual Earthworms in 60g Wet Weight of Digested and Undigested Solids

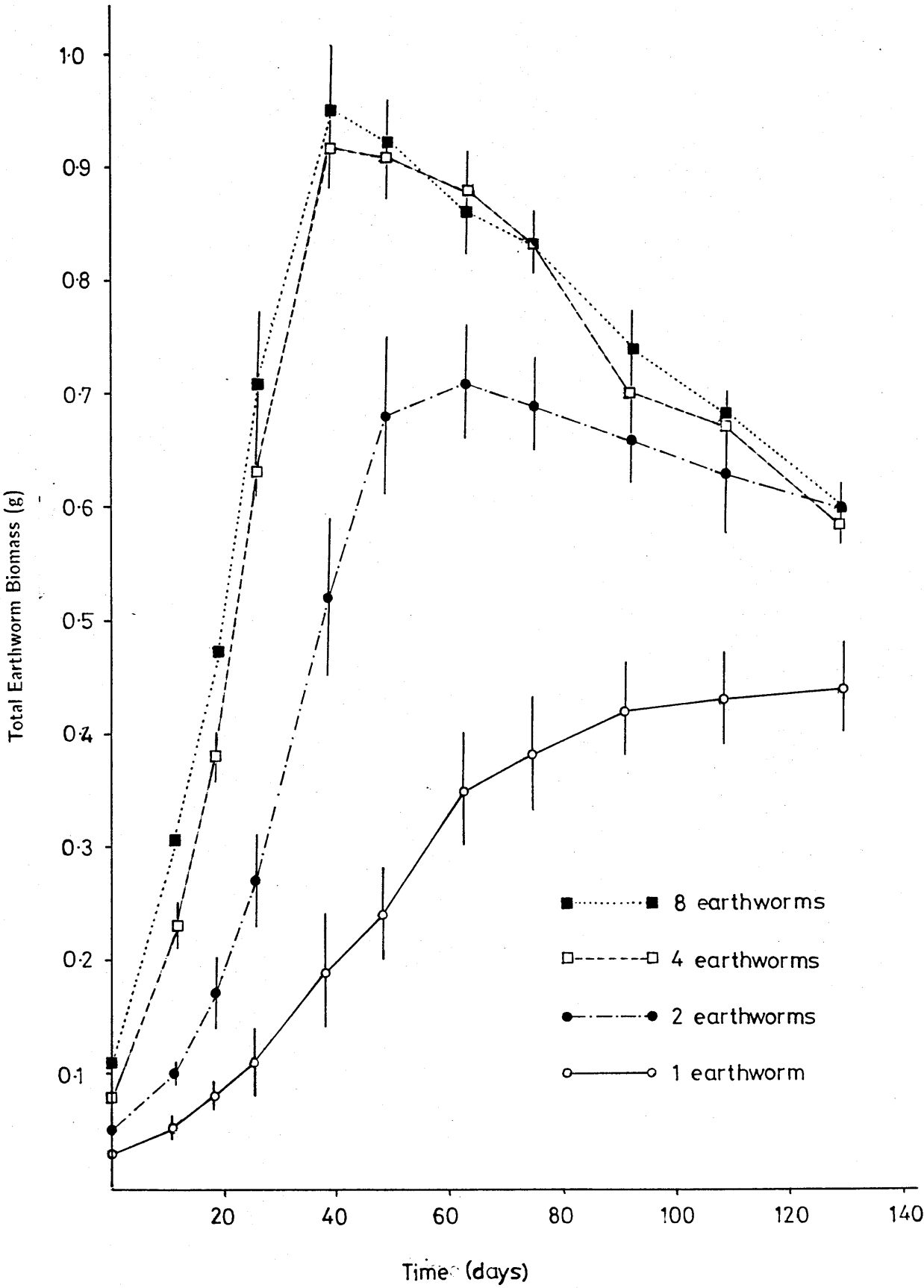
Graph 4.2.3.2. The Onset of Sexual Maturity
in Earthworms Grown Individually in
Digested and Undigested Solids



Graph 4.2.3.3. The Growth of 1,2,4 and 8 Earthworms in 60g of Undigested Solids



Graph 4.23.4. The Growth of 1,2,4 and 8 Earthworms in 60g of Digested Solids



4.2.4) Discussion of Results.

Using Students t-test, the results show that after 48 days the mean weight of earthworms growing in digested solids is significantly higher than those in undigested solids, whose final weight was almost half that of earthworms in digested solids (0.32g compared with 0.57g).

Approximately 50% of earthworms in digested solids became sexually mature after 10 weeks, peaking at 65% after 14 weeks. The presence of a clitellum was used to indicate the onset of sexual maturity. In comparison only 5%, or one of the 20 earthworm replicates in undigested solids became mature over the 20 week span of the experiment.

The results indicate that the mean weight of earthworms in undigested solids did not rise high enough to initiate the onset of sexual maturity. The one earthworm that did become mature after 140 days weighed 0.77g. The mean earthworm weight on attaining sexual maturity in digested solids was 0.41 ± 0.03 g, compared with a maximum mean weight of 0.32g achieved by earthworms in undigested solids. This indicates an inhibition of earthworm growth in undigested solids.

Neuhauser *et al* (1980) achieved a maximum *E.fetida* weight of approximately 2.4g in activated sludge and 1.9g in horse and cow manure. This was achieved with 250g of the appropriate solids that was replaced every week. This was the maximum possible weight that could be achieved on an unlimited supply of food, and cannot be compared with the results obtained in this section. However in another experiment Neuhauser *et al* (1980) grew individual *E.fetida* in limited rations of organic waste achieving maximum weights of 0.2g, 0.3g, 0.5g, and 0.6g on 5, 10, 15, and 20g respectively of activated sludge with a moisture content of 89%. This represents an average conversion efficiency of 3.3% wet weight to wet weight, compared with a figure of 0.95% for digested solids and 0.53% for undigested solids achieved in this experiment. Here is evidence to suggest that

both digested and undigested solids have an inhibition effect on the growth rate of *E.fetida* within it, with undigested solids displaying this to a greater degree.

The experiment was designed to keep the temperature and moisture content identical for the two treatments. The levels were at an optimum calculated in experiments at Rothamsted experimental station (unpublished data). The initial pH levels for both solids were similar for both treatments, whilst the concentration of NH_3 and NH_4^+ ions were very low. However the ionic conductivity levels in both solids were high, being greater in undigested than digested solids.

The results from graphs 4.2.3.3 and 4 support the work of Edwards (1983) who, in culturing earthworms at varying stocking densities in potato wastes concluded that overall productivity, as measured by earthworm weight, was highest at highest stocking densities. In this experiment 8 earthworms in digested solids grew only marginally better than 4 earthworms, achieving a maximum weight of 0.95g. Eight earthworms reached the highest weight in undigested solids but at a lower peak of 0.775g.

The graphs show different patterns of growth for earthworms in digested and undigested solids. Firstly, whereas the weight of four and eight earthworms in digested solids peaked at approximately 0.95g, eight earthworms in undigested cattle solids reached a plateau of approximately 0.775g for 25 days before a sharp drop in weight. Secondly, two earthworms in digested solids reached a peak after 62 days, but in undigested solids 1, 2 and 4 earthworms continued a slow increase in weight for the 128 day duration of the experiment.

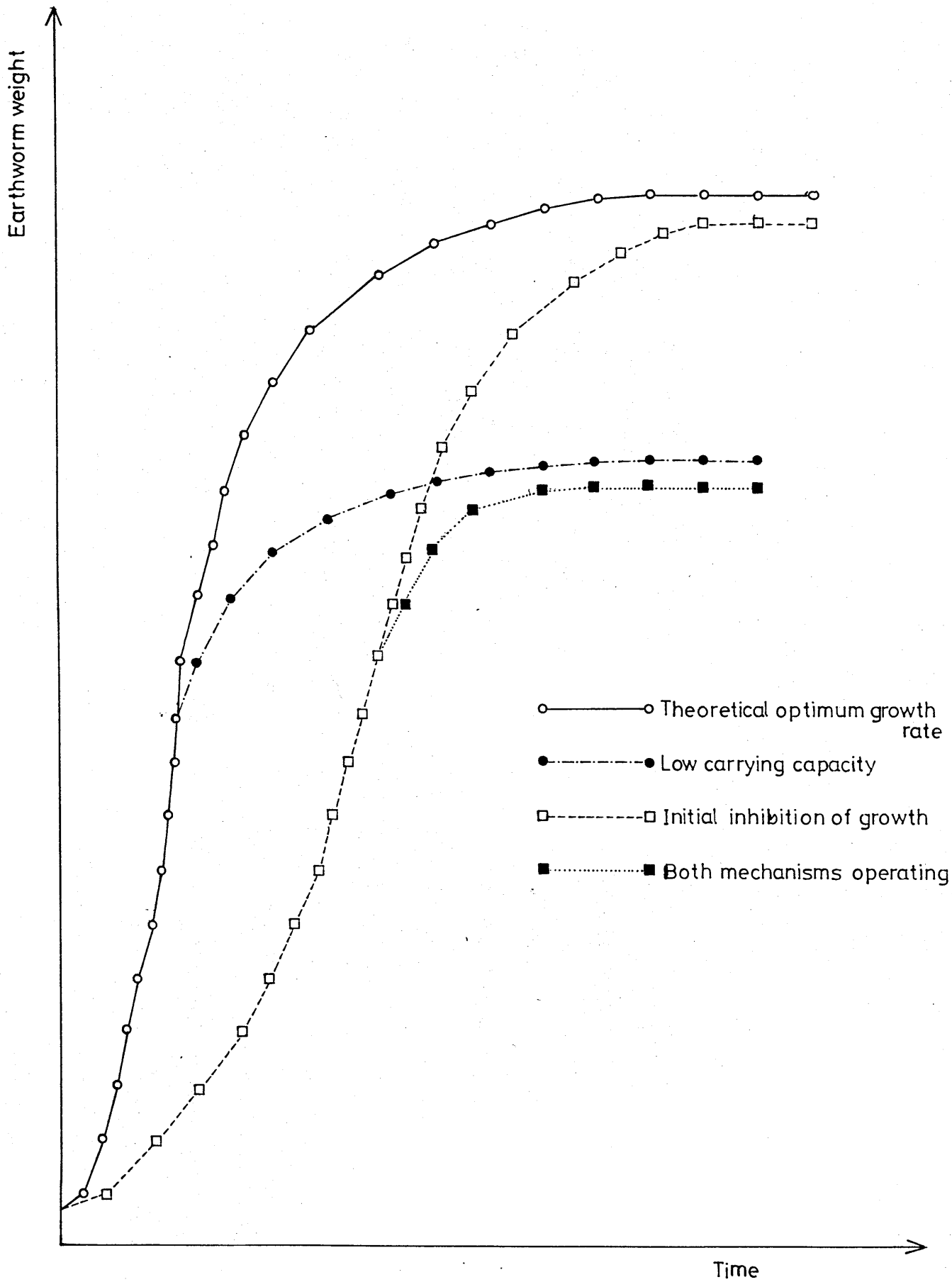
The results therefore show that as with individual earthworms some factor within undigested solids caused a lower rate of growth than that observed in digested solids, but for 8 earthworms there was less growth inhibition. It is suggested that the physical presence of 8 earthworms within a limited mass of undigested solids brought about an

alteration in the factors causing inhibition. This may have been an aeration effect caused by the earthworms borrowing activities, or a change catalysed by the earthworm gut environment on the ingestion of solids. Although this activity reduced the inhibition of earthworm growth, the maximum weight recorded was still lower than digested solids, indicating that undigested solids have an overall lower carrying capacity as well as having an inhibitory effect on growth rate. The dramatic fall in weight for 8 earthworms after the plateau maximum had been reached supports the view that undigested solids have a lower earthworm biomass carrying capacity.

Although the experiment does not indicate how earthworm growth inhibition is operating, the results indicate that there are two forms of inhibition occurring; a reduction in the initial rate of growth, and also a decrease in the maximum earthworm weight achieved when compared with the published data (Hartenstein 1980). This suggests that non-optimum environmental conditions within the solids are inhibiting the growth rate within the solids, and the nutritional value of the solids, as defined by the maximum weight gain is also reduced. In both cases the effect is greater for undigested solids than digested solids.

From the above two mechanisms for the inhibition of earthworm growth can be proposed, the first causing inhibition in the rate of growth, especially in the early stages for young earthworms, and the second being a reduction in the nutritional value of the waste, which reduces the maximum weight achieved without necessarily affecting the initial rate of growth. Such models of inhibition can be represented graphically as in graph 4.2.4.1.

Graph 4.241. Theoretical Models of Earthworm Growth Inhibition

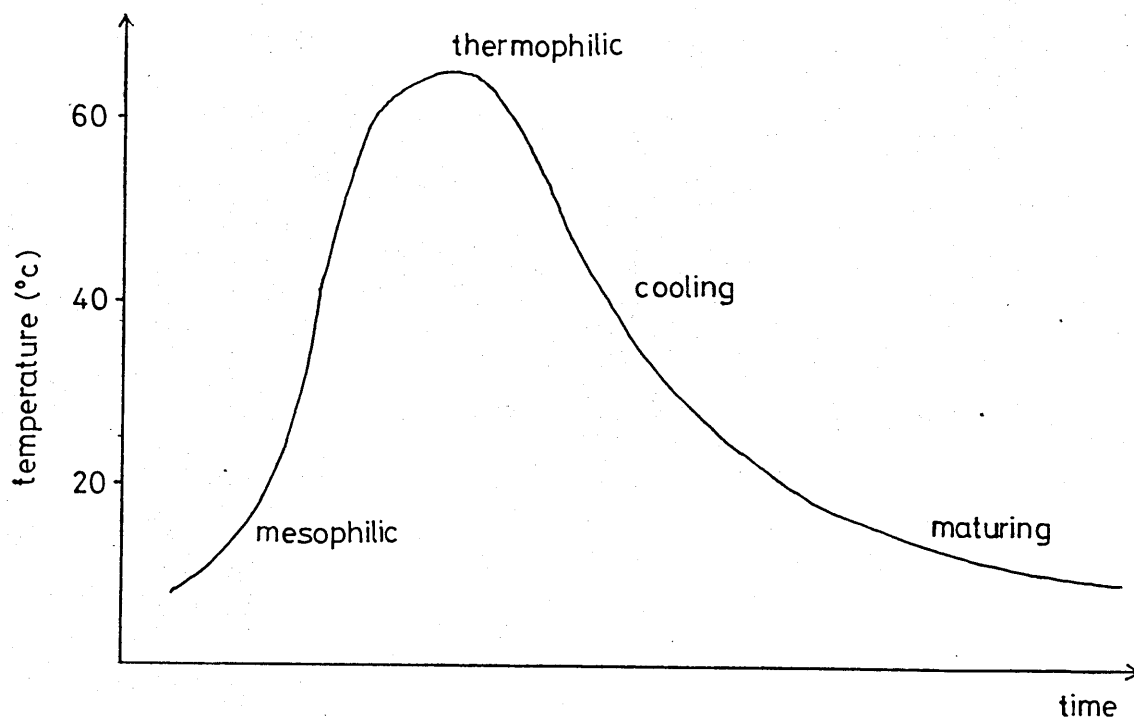


4.3) The Effects of Maturing Digested and Undigested Solids for Eight Weeks on the Growth of *E.fetida*.

4.3.1) Introduction.

In earlier field experiments, a possible cause of the observed low growth rates was related to the pre-treatment of the solid after mechanical separation and prior to inoculation by earthworms. In an initial field experiment two possible methods of pre-treatment were used and their effect on earthworm growth measured. In this section the length of time after separated solids have become acceptable to earthworms is studied. The hypothesis under consideration is whether over long time periods some deleterious change occurs within the separated solids as part of the decomposition process

Gray and Biddlestone (1971) divide the composting process into four phases, mesophilic, thermophilic, cooling and maturing, which can be seen plotted against temperature below;



From the hypothesis above there may be microbially induced processes occurring in the maturation phase of composting which are deleterious to earthworm growth. Firstly, the changes may reduce the palatability of the solids, and so inhibit earthworm ingestion in the gut. Secondly, the nutritional quality of the solids may be reduced, so that although the ingestion of the solids is unaffected, not enough nutritional material can be extracted from the solids to support normal earthworm growth. Finally, the changes occurring over time may have changed environmental conditions within the solids to make them sub-optimal for *E.fetida* and so adversely affect earthworm growth.

Therefore, a simple duplicate experiment was carried out in the laboratory using digested and undigested solids left to age for a period of 8 weeks once acceptable, before being used to support the growth of 1 or 8 *E.fetida*. The results are linked to a physico-chemical analysis of the aged solids in order to examine the effects of aging.

4.3.2) Materials and Methods.

As in section 4.2.2, except that the solids were used eight weeks after the point of initial earthworm acceptability, and then analysed to adjust the moisture content. 60g fresh weight of undigested solids was placed in each of 20 plastic containers, half inoculated with individual *E.fetida* hatchlings and half with eight hatchlings. The process was then repeated for digested solids.

4.3.3) Results.

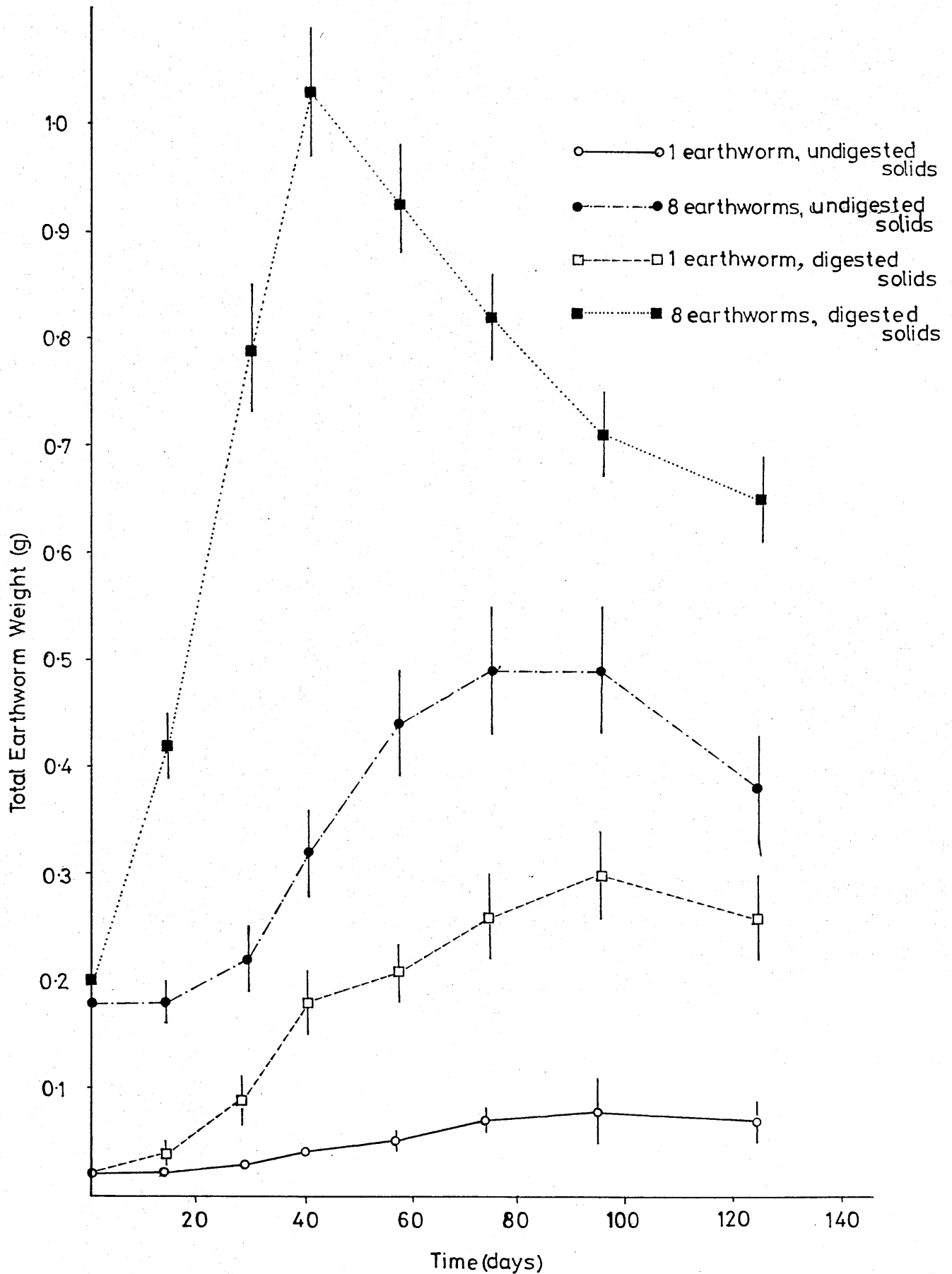
Graph 4.3.3.1 shows the growth of 1 and 8 earthworms in digested and undigested solids, and table 4.3.3.1 shows the results of physico-chemical analyses on the aged solids.

Table 4.3.3.1.

Changes in Physico-chemical Characteristics of Undigested and Digested Solids on Maturation for Eight Weeks.

Variable	Undigested Solids Acceptable	Undigested Solids Matured	Digested Solids Acceptable	Digested Solids Matured
T.S.(%)	23.7	26.3	25.2	28.1
pH	8.7	8.3	8.7	7.9
Ionic Conductivity (μS)	5350	4350	4050	3900
NH ₃ -N (mg/g dry wt)	0.01	<0.01	0.02	<0.01
NH ₃ -N and NH ₄ ⁺ -N	0.01	<0.01	0.02	<0.01

Graph 4.3.31. The Growth of 1 & 8 Earthworms
in Undigested and Digested Solids Matured
for 8 Weeks



4.3.4) Discussion of Results.

A marked difference has emerged between the results of earthworm growth in digested and undigested solids which have been allowed to age. Taking the results from digested solids first, the growth of 8 earthworms was almost exactly similar to those results obtained in section 4.2 at the same inoculation density. Individual earthworm growth was less, peaking at 0.3g live weight per earthworm compared with 0.44g in the earlier study.

For undigested solids, both individual and 8 earthworms reached lower maximum weights than those recorded in section 4.2, 0.49g and 0.08g compared with 0.77 and 0.33g respectively.

In addition some of the replicates suffered mortalities. Three in ten earthworms died in the individual earthworm trial in undigested solids, and one in ten in digested solids. A similar percentage mortality rate was observed in the growth trials with 8 *E.fetida*, 25 from 80 (31%) in undigested solids and 8 from 80 (10%) in digested solids. If such results were not obtained by chance then it seems that both digested and undigested solids have put environmental stress on the earthworms which can be measured quantitatively through earthworm mortalities.

Unfortunately the physico-chemical analyses provide little direct information on the processes occurring in the 8 week maturation process that the separated solids underwent. The physico-chemical variables are all within broad limits of acceptability outlined in chapter one, although, as noted earlier there is little data on how a combination of physico-chemical factors effect earthworms. However an indication of the microbial and chemical activities can be obtained from the literature.

Yung Chang (1967), studying the composting of wheat straw with added ammonium

nitrate found the material lost half its dry weight in the first 34 days, with the losses almost entirely due to cellulose and hemi-cellulose breakdown. In the following 131 days, only 4% additional weight loss was recorded, indicating that the initial phases of composting are responsible for the vast majority of carbon breakdown, and that such processes are less significant in the aging or maturation undergone by solids in this experiment.

The most important event that occurs in the aging or maturation of organic materials is the process of humification, whereby the fungal breakdown products of lignin and associated compounds are polymerised into a family of large stable aromatic compounds known together as humus (Grushnikov and Antropova 1975, Christman and Oglesby 1971). This is a relatively slow process which is still poorly understood, but is a fundamental aspect of decomposition. Its effect upon earthworms is difficult to determine, as earthworm activity proceeds hand in hand with humification processes. Lignin cannot be used as a source of nutrition (Neuhauser *et al* 1978) and so its incorporation into humus like molecules is not going to make matured solids any nutritionally poorer and so reduce maximum earthworm biomass. There is also no evidence in the literature to suggest that polymerised humic molecules are toxic to earthworms, and given the intimate relationship between earthworms such as *E.fetida* and the decomposition and humification process this makes biological sense.

Clark (1969) describes how soil microbes interact with each other in a process known as suppression, whereby one organism can effect another through means of a deleterious change in the environment, either through competition or antibiosis. Such interactions have been postulated to occur in the final stages of composting by Burman (1961).

If such antagonistic relationships between microbes are occurring in maturing compost as the competition for dwindling resources intensifies, then it may affect earthworms within the waste in one of two ways. There may be a direct antibiotic effect on the earthworms themselves, the intense competition and the subsequent reduction in microbial numbers

may remove the groups of micro-organisms being utilised by *E.fetida* for nutrition.

Another process occurring alongside humification is the loss of nitrogen from the separated solids on maturation. Ammonia will be continually formed at low concentrations by the metabolic processes of microbes, and this will be at risk of being lost through volatilisation, especially in the alkaline conditions of the digested and undigested solids in the experiment.

Two processes will act against this $\text{NH}_3\text{-N}$ loss. Firstly, there is the possibility that populations of nitrifying bacteria may develop, converting ammonia to nitrate, which will not be lost from the non-draining system used in this experiment. Secondly ammonia may be synthesised into the microbial biomass as organic molecules. As detailed earlier there will be intense competition for resources in maturing compost including N and so any released through large numbers of microbial mortalities is likely to be used and incorporated immediately.

One other possible apparent loss of nitrogen comes from the chemical reaction of ammonia with humic molecules. Wieringa (1964) noted that large amounts of ammonia reacted with the humus compounds of sphagnum peat in such a way that it became biologically immobile. Tsutsuki and Kuwatsuka (1978) found that between 0.5 to 3% of humic acids were made up of non-hydrolysable nitrogen in a stable, non-reactive state. Therefore there is theoretical evidence to suggest that nitrogen may be locked in humic complexes on the maturation of composted separated solids, coupled with a loss of N through volatilisation, leading to an overall reduction in earthworm available N.

In the original results one anomaly remains unexplained. The graphs show that 8 *E.fetida* cultured in digested solids grew as well as those in non-aged digested solids (section 3.3), but individual earthworms in digested solids showed signs of suppression of growth for both 1 and 8 *E.fetida* in matured undigested solids inhibition of growth was seen. With the only difference between the set-ups being the number of earthworms present, it is

likely that the results are due to an effect stimulated by the physical activity of the earthworms in the experiments. The action of eight hatchlings moving through the separated solids may have been to aerate the solids and stimulate microbial activity, producing a ill-defined positive feedback response to improve conditions within the mature solids.

There was an observed difference in the physical appearance of the matured separated solids. The undigested solids had a less well defined physical structure which deteriorated still further on maturation. Microbial activity on thermophilic composting utilises oxygen very quickly and can create pockets of anaerobiosis.

If anaerobic microsites develop within the undigested solids these may encourage anaerobic denitrifying bacteria, leading to a loss of nitrogen as N_2 (Fillery 1983). In addition, many high energy molecules broken down in the anaerobic digestion process, such as proteins, volatile fatty acids and carbohydrates, will be initially present in undigested solids. The demand for oxygen in the break down of such molecules increases the chances of anaerobic sites developing, which can lead to the incomplete decomposition of such high energy compounds, leading to putrefaction and possible earthworm toxin production.

4.4) Conclusions to the Chapter.

Although the results of individual and multiple earthworm growth trials have produced lower growth rates than quoted in the literature, the extrapolated increases are higher than those observed in field trials. Some scaling up factor has obviously adversely affected the results of field scale trials.

In these laboratory scale studies digested solids have supported earthworm growth better than undigested solids, confirming earlier field scale trial results. Possible reasons for such a difference are considered.

High stocking densities have been shown to decrease the inhibition effect of Bore Place solids, and this has relevance for the interpretation of field scale trial results. Finally, two possible mechanisms for the overall low growth rates in Bore Place solids are considered.

Chapter Five.

Comparison of Bore Place Solids with Similar Material from Other Sites.

5.1) Introduction.

Field scale trials in chapters one to three and laboratory experiments in chapter four have not produced results in accordance with published data. The general conclusion drawn from this work is that Bore Place solids do not represent an optimum substrate for earthworm growth. Several possible mechanisms for why this may be so have been discussed. However, in order to test the hypothesis that Bore Place solids do not make a good substrate for earthworm growth, the material needs to be compared with similar materials from other sites, to avoid the problems of studying Bore Place material in isolation, and to obtain an objective evaluation of how Bore Place solids rate as a substrate for earthworm growth.

To achieve this material from three sites is examined, Bore Place, The Bernard Weitz Centre at the National Institute for Research into Dairying (NIRD) now part of the Institute for Grassland and Animal Production, and Oaklands Agricultural College near St. Albans.

By examining how the waste management and treatment methods of the three sites vary, and the effect upon the physico-chemical constituents of the separated solids, subsequent earthworm growth rate data can be interpreted to modify and improve the theoretical mechanisms of earthworm growth inhibition.

5.1.1) Site Descriptions.

Bore Place. See Section 2.1.1.

NIRD.

Slurry of approximately 7% total solids (T.S.) was collected from a herd of 400 lactating British Friesian cows, fed on a maize silage based diet. The cattle were housed throughout the year and bedded on sawdust. The cubicle shed passageways were scraped twice daily using a tractor into a below ground channel which by means of mechanical scrapers discharged the slurry and some parlour yard washings into a 10cum reception pit. A submersible electric pump was used to mix the contents of the pit and feed slurry to the mechanical separator. This was a two stage roller press machine, available commercially from Farrow Irrigation ltd and mounted on a 4m high gantry to allow drainage of the liquid fraction of slurry into a storage tank, and the solid fraction to be loaded onto a trailer. The separator produced a liquid of 4% T.S. and a solid of approximately 15% T.S. depending on its settings. It can be seen in photograph 5.1.1.1.

Photograph 5.1.1.1.

The Brush and Roller Farrow Separator
in Use at NIRD.



Oaklands Agricultural College.

The 97ha college farm provides grass and silage for a dairy herd and ewe flock. The 120 pedigree British Friesian herd is housed in cubicles over winter on chopped straw, and the slurry produced held in an above ground store before undergoing mechanical separation in an prototype Farrow two-stage roller press.

5.1.2) Materials and Methods.

Freshly separated solids were collected from each of the three test sites and transported back to the Open University. Before use as an earthworm growth medium the solids were thermophilically composted as described in section 6.1.3.

5.1.3) Physico-Chemical Analyses of Solids During Pretreatment.

At five points in time during the six week pretreatment period samples of the four types of waste were taken for physico-chemical analyses of the following parameters;

Total solids (%).

The fresh sample was weighed, dried in an oven set at 105°C overnight and reweighed after being allowed to cool down in a desiccator.

Volatile solids (%).

Oven dried solids were weighed and then placed in a furnace at 550°C for a minimum of two hours before cooling in a desiccator and reweighing.

pH.

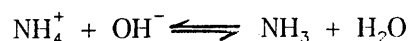
An EIL pH probe was placed directly into fresh samples, and the mean of a minimum of three readings taken at different positions within the mass of waste and recorded on a Digital water analyser was taken as the pH value.

Ionic Conductivity.

For this set of readings a different method of analyses was adopted to those of section 2.5.4. 10cm³ of freshly sampled solids were loaded into a conductivity cell with a cell constant of one. The solids were packed into the cell at a constant density, and the mean reading of a minimum of three measurements, recorded on a Digital water analyser, was taken as the ionic conductivity, measured in $\mu\text{S}/\text{cm}$. Although the results obtained by the use of this method are not directly comparable with those obtained in section 2.5.4, the method adopted was less time consuming.

Ammonia and Ammonium ion Analysis.

An EIL NH₃ probe was connected to a Digital water analyser and used to measure NH₃ and NH₄⁺ ion concentrations in an aqueous solution, 4g wet weight of fresh solids thoroughly mixed with 20ml distilled water. Free ammonia (NH₃) was measured directly from the aqueous extract. Ammonia and ammonium ions were measured after the addition of a 1M sodium hydroxide (NaOH) solution, making up 10% of the sample. This shifts the NH₄⁺ ions into the molecular state where they could be detected by the EIL probe.



For further information see Byrne and Power (1974)

Total Kjeldahl Nitrogen.

The macro-digestion method was adopted, using a method modified from MAFF (1981). 5g fresh weight of separated solids were placed in a boiling tube with two anti-bumping balls, 23ml of concentrated sulphuric acid (H_2SO_4) and 7ml concentrated digestion reagent containing selenium. Also 6g sodium sulphate (Na_2SO_4), 0.1g of copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), and 0.05g selenium were added in the form of preweighed tablets.

The solution was refluxed overnight until clear, and then distilled on a Buchi distillation unit. 100ml of distilled water was added to the boiling tube, followed by 100ml 12.5N NaOH. Steam was then passed through the solution and 200ml of distillate was collected in a beaker containing 25ml 2% boric acid solution, and three drops of SHER indicator.

The distillate was titrated against 'Analar' 0.1M H_2SO_4 , and kjeldahl nitrogen calculated from the following formula;

Total Kjeldahl Nitrogen (TkN)

$$= M \times V \times 2 \times 1.4 / W$$

where;

M = molarity of the titrate, V = volume of the titration in ml

W = fresh weight of the original sample, 2 = normality of H_2SO_4

Total Organic Carbon.

A modified Walkley-Black method was used to calculate total organic carbon (Hesse 1971). On the assumption that separated cattle solids contain very little quantities of inorganic carbon this value can be taken as the total carbon value in the calculation of the carbon/nitrogen value.

0.05g of oven dried material, ground to pass through a 1mm sieve, is placed in a 500ml conical flask. To this is added 20ml M/6 potassium dichromate (24.52g in 500ml) and 20ml of concentrated sulphuric acid. The flask was swirled for thirty seconds and then placed on an asbestos mat and allowed to cool for thirty minutes. When cool 200ml of distilled water is added to the flask, along with 10ml of concentrated orthophosphoric acid, and allowed to cool for a further ten minutes. The flask contents are then thoroughly mixed and 10ml was then removed and placed in a glass petri-dish. The sub-sample was then titrated with 0.5M ammonium iron II sulphate solution, with the end point being a colour change from yellow to green. The recommended indicator did not help to define the end point and the colour change was difficult to observe when carried out with 250ml of solution in the flask.

The percentage organic carbon value of the sample was carried out as follows;

% oxidisable organic carbon, Walkley Black, uncorrected

= (Blank titre-actual titre) x 25 x 0.3 x M /Wt of solid used

Where;

25 = dilution factor (10ml taken from 250ml)

M = molarity of ammonium iron II sulphate solution.

Although an easy and convenient means of measuring organic carbon the Walkley-Black method does not recover 100% of the carbon present (Walkley 1947). An arbitrary conversion factor is therefore employed to enable the results to be expressed as total organic carbon, normally taken as 1.33 or a 75% recovery rate (Hesse 1971).

5.2) Earthworm Growth Trial: Materials and Methods.

All solids used for earthworm growth were first adjusted to 85% moisture content before being weighed out into plastic containers, as in section 2.3.2, except 60g of solids was used on this occasion. Hatchlings were pre-sorted by hand from an active population of earthworms in separated solids and used as the inoculum for the containers. The weight range of the hatchlings used was between 0.01 and 0.03g.

Once inoculated the containers were placed in an incubator at $20 \pm 1^\circ\text{C}$, removed every 7-14 days and weighed as in section 2.3.2. At the same time the earthworms were examined for the presence or absence of a clitellum, used as a measure of sexual maturation. 15 replicates of each run tabulated in section 3.2.1 were set up, and the results obtained expressed as the mean of the replicates, and plotted graphically.

5.2.1) Results.

Tables 5.2.1.1, 2, and 3 show changes with time during the composting of separated solids from the three sites in terms of physico-chemical parameters.

Graphs 5.2.1.1 and 5.2.1.2 show the growth of individual and eight earthworms in separated solids from the three sites, treated in an identical manner.

Table 5.2.1.1.

Physico-chemical Analysis of Thermophilically Composted
Bore Place Undigested Solids.

Variable	0 days	5 days	12 days	34 days	45 days
T.S. (%)	19.4	20.9	20.9	22.7	21.9
V.S.(%)	85.5	83.1	87.7	78.2	77.5
pH	9.0	8.3	8.2	8.3	8.5
Ionic Conductivity (μ S/cm)	3370	5630	5920	6930	7180
NH ₃ -N (mg/g d wt)	0.40	0.12	0.07	<0.01	<0.01
NH ₃ -N and NH ₄ ⁺ -N (mg/g d wt)	3.07	1.94	0.67	0.03	0.02
Total Kj-N (%)	3.5	3.4	2.9	2.7	2.3
Total Organic Carbon (%)	73.8	55.9	51.9	49.9	43.9
C/N Ratio	21.1	16.4	17.9	18.5	19.1

Table 5.2.1.2.

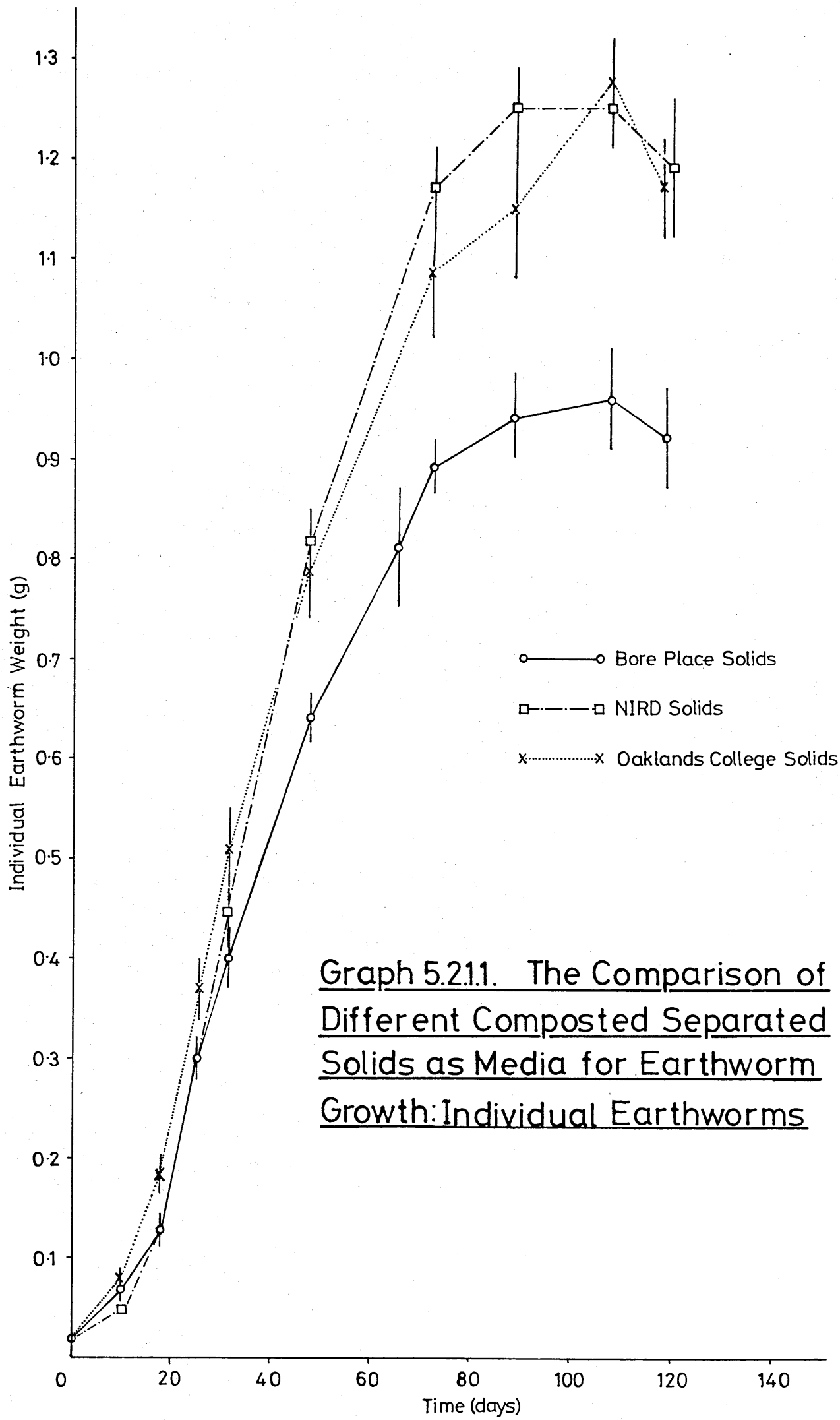
**Physico-Chemical Analysis of Thermophilically Composted
NIRD Solids.**

Variable	0 days	5 days	12 days	34 days	45 days
T.S. (%)	16.0	16.0	16.0	15.8	16.2
V.S.(%)	90.9	79.5	68.9	87.4	74.3
pH	7.6	8.1	7.9	8.3	8.6
Ionic Conductivity (μ S/cm)	3100	3130	3260	4110	4230
NH ₃ -N (mg/g d wt)	0.17	0.11	0.03	0.01	<0.01
NH ₃ -N and NH ₄ ⁺ -N (mg/g d wt)	3.97	1.69	0.48	0.26	0.21
Total Kj-N (%)	3.3	3.0	2.6	2.0	1.9
Total Organic Carbon (%)	73.8	62.5	61.2	61.8	59.9
C/N Ratio	22.4	20.8	23.5	30.9	31.5

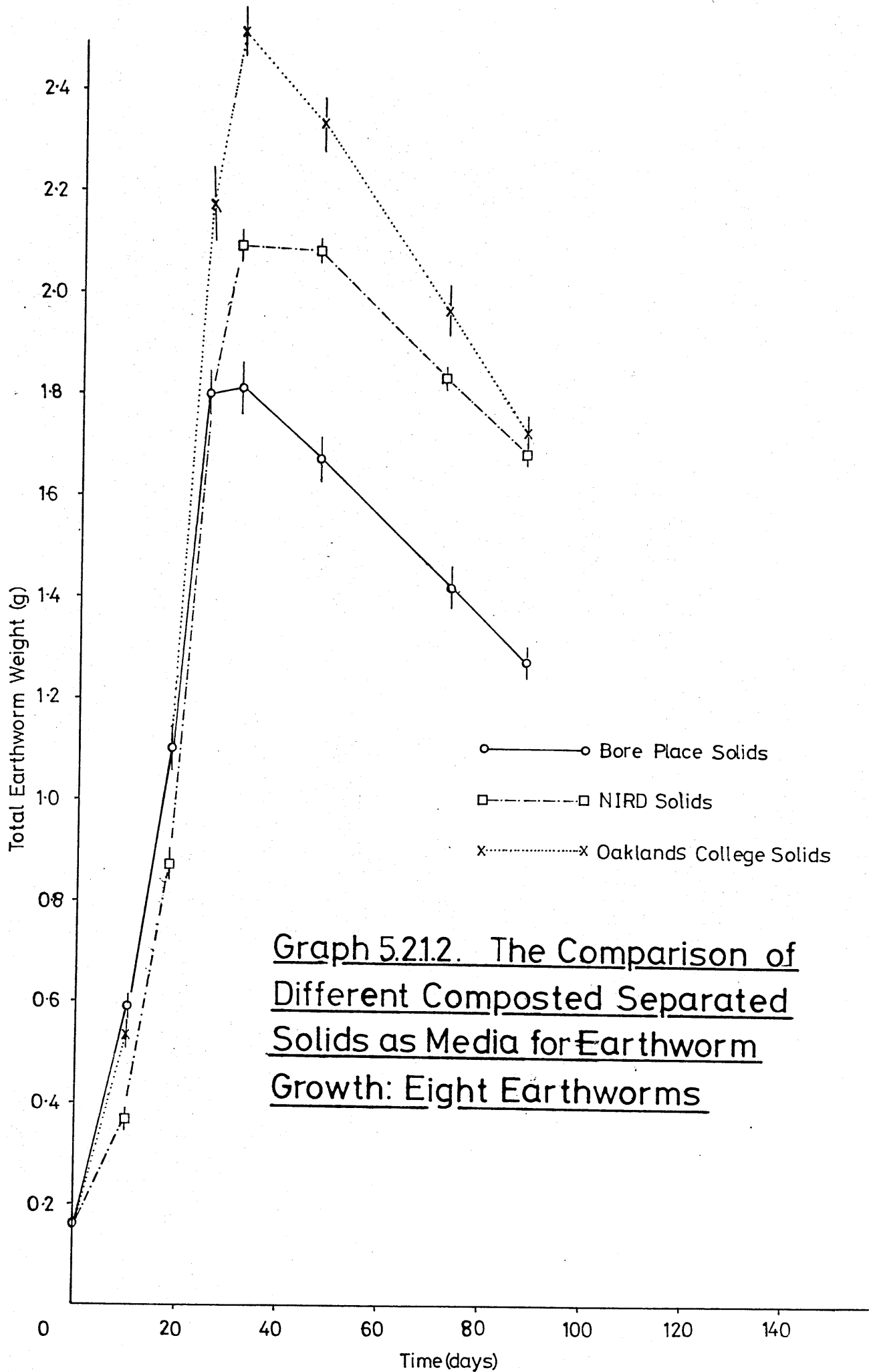
Table 5.2.1.3.

**Physico-Chemical Analysis of Thermophilically Composted
Oaklands College Solids.**

Variable	0 days	5 days	12 days	34 days	45 days
T.S. (%)	17.1	16.4	17.3	17.0	17.4
V.S.(%)	82.0	80.1	74.2	77.7	75.7
pH	8.1	8.0	8.0	8.2	8.3
Ionic Conductivity ($\mu\text{S}/\text{cm}$)	4650	4620	4780	7270	7310
$\text{NH}_3\text{-N}$ (mg/g d wt)	0.12	0.10	<0.01	<0.01	<0.01
$\text{NH}_3\text{-N}$ and $\text{NH}_4^+\text{-N}$ (mg/g d wt)	4.91	0.68	0.07	0.03	<0.01
Total Kj-N (%)	3.8	3.5	3.0	3.2	2.9
Total Organic Carbon (%)	71.8	61.8	67.8	55.9	51.9
C/N Ratio	18.9	17.7	22.6	17.4	17.9



Graph 5.21.1. The Comparison of Different Composted Separated Solids as Media for Earthworm Growth: Individual Earthworms



Graph 5.21.2. The Comparison of Different Composted Separated Solids as Media for Earthworm Growth: Eight Earthworms

5.2.2) Discussion of Physico-Chemical and Earthworm Growth Results.

The physical, chemical and biological nature of the solids from the three sites depends on the following factors;

The age and physiological status of the cattle.

The diet of the cattle.

Methods of collection and storage of the slurry.

Type and settings of the separator.

The storage and treatment methods of the solids.

The earthworm growth pattern will be in direct response to the physico-chemical characteristics of the solids generated by the above factors. It is therefore worth discussing the differing growth patterns shown by earthworms growing in the three types of solid, and relating this to the physico-chemical factors recorded in tables 5.2.1.1, 2 and 3.

Graph 5.2.1.1 of individual earthworms cultured in 60g wet weight of solids, shows very similar initial rates of biomass increase for the first 30 days, but the maximum weight of earthworms in Bore Place solids peaks at a much lower weight than earthworms in Oaklands college or NIRD, so that after day 47 until the end of the experiment, the mean weights of earthworms in Bore Place solids were significantly different to both those in NIRD and Oaklands college solids using Students t-test. After 10 and 18 days the mean earthworm weight in Oaklands college solids was significantly higher than for NIRD solids but after 18 days there was no significant difference between them.

Students t-test was also used to compare the mean earthworm weights at varying times for 8 earthworms per 60g of solids. On day 10 the mean weight of earthworms in Bore Place undigested solids was significantly greater than those in NIRD solids at the 5% level of significance. On day 18 both Oaklands college and Bore Place solids produced mean weights that were significantly greater than NIRD solids. From day 25 until the

end of the experiment Oaklands college mean earthworm weights were significantly higher than those in Bore Place, and from day 31 so were those of NIRD solids. Oaklands college solids also continued to produce significantly higher mean earthworm weights than NIRD solids from day 18 to day 47. Most importantly, at the time of peak growth, the measured earthworm weights in Oaklands solids were significantly higher than those in NIRD solids which in turn were significantly higher than those in Bore Place solids. With 8 earthworms per 60g of solids producing greater competition for resources within each solid, one would expect the differences between the solids to be more pronounced.

Referring to the theories of earthworm growth inhibition described in section 4.2.4, the results here indicate that there is no inhibition of the initial growth phase of earthworms in Bore Place Solids but waste from this site is not able to support as high an earthworm biomass as NIRD and Oaklands solids, especially at high stocking densities. The lower carrying capacity mechanism could therefore be described as operating.

The difference between earthworm growth in Bore Place solids and the other two sites is not great enough to explain the poor growth and reproduction observed in earlier populations of earthworms cultured in Bore Place solids. This indicates that factors other than Bore Place solids simply being a poor substrate for earthworm growth are involved in producing the observed results, although the measured carrying capacity of Bore Place solids is a contributory factor.

Relating the physico-chemical characteristics of the solids from the three sites with the observed earthworm growth is not easy, as there are no obvious differences between the solids that could account for the different patterns of earthworm growth. The main physico-chemical difference between the three solids after 45 days of composting is the low percentage organic carbon value for Bore Place solids and the low ionic conductivity and high C/N ratio of the NIRD solids. Neither of these two observations alone could account for the observed differences between the various separated solids ability to support

earthworm growth.

Variations in the waste produced by differences in the site should also be taken into account. These were the cattle diet, the bedding material used and the types of separator at each site, as detailed in section 5.1.2.

Dietary considerations will have the major influence on the form of solid produced, as faecal material will predominantly be degraded components of ingested foodstuffs. The relationship between diet and animal waste characteristics cannot be easily summarised.

The effects of differing cattle bedding material on the form of waste produced are more limited. The size of the bedding material will have a great influence on the final particle size of the separated waste, affecting the surface area exposed for microbial attack, and also the amount of air space within the solid, and thus its aerobicity. Oaklands college solids with chopped straw as the bedding material contained the coarsest material, followed by the woodchips of Bore Place solids and NIRD solids containing a high proportion of woodshavings, producing the densest, least aerobic of the three solids.

The bedding which becomes incorporated into the separated wastes represents the largest single source of fibre within the solids and this defines to a great extent the forms of fibre that will be present. The wood based bedding of the cattle waste at NIRD and Bore Place will contain a higher proportion of lignin than the straw of Oaklands agricultural college, and wood based bedding will also be an unknown mix of hard and softwoods, the latter possibly containing harmful phenolic-like resins.

In terms of the differing effects of the various bedding materials on earthworm growth, the statistically higher earthworm weights achieved in Oaklands solids compared with NIRD and Bore Place fit into the expected pattern. The large particle size of the straw bedding material helps maintain aerobicity within the solids, and the lower proportion of

resistant lignin-related fibre allows greater breakdown and utilisation of the carbon as a source of energy.

However, comparison between earthworm growth in Bore Place and NIRD solids does not produce results that can be related to the type of bedding used. Both are based on wood and the main difference between them is the particle size. It has been argued previously that the wood shavings of NIRD solids may tend to more anaerobic conditions, and thus reduced earthworm growth. This has not been borne out by the significantly greater recorded earthworm weights of NIRD solids compared to Bore Place solids.

The recorded growth patterns in the solids from the three sources cannot therefore be linked directly to any one physical or chemical variable that differs between them, supporting the view that there must be subtle interaction between the physico-chemical factors that make up the solids, both those measured and those not, to produce the final substrates which influence the observed growth responses in earthworms. The nature of the earthworm growth curves for both individual and eight earthworms is commensurate with this, indicating no direct inhibition of growth by one or more physico-chemical factors, but rather a variety of inter-related responses which together act to reduce the carrying capacity of Bore Place solids in relation to Oaklands and NIRD solids, and to a lesser extent, NIRD solids to Oaklands solids.

That no clear relationship between the measured growth curves and any physico-chemical factors within the waste places an emphasis on the role of biological factors within the solid which will effect earthworm growth, especially the diversity and activity of micro-organisms within the solids. Slight differences in microbial populations within solids from different sites may have an important influence on physico-chemical factors that will in turn effect earthworm growth.

In conclusion, the results indicate the solids produce a range of earthworm growth responses

dependent not on any particular physico-chemical or biological characteristic of the solids, but rather a complex interaction between the factors within the waste which produce a sliding scale of earthworm growth responses. Therefore relating this work back to the earlier field scale trials, the results indicate that Bore Place solids have no intrinsic qualities that rule it out as a medium for supporting earthworm growth, but that the conditions prevailing at Bore Place produce a solid whose characteristics are less well suited to earthworm growth than materials from other sites, including those reported in the literature.

Chapter six.

Changes in the Physico-Chemical Characteristics of
Separated Cattle Solids upon Thermophilic Composting
and Mesophilic Aging and its Effects upon *E.fetida*.

6.1) Introduction.

In section 2.3.4 it was seen that allowing a compost to mature over a period of eight weeks influenced earthworm growth and reproduction. This serves to highlight the importance of separated solid pretreatment prior to inoculation and indicates that the way in which a solid is treated prior to earthworm culture can have important effects. In practice solids are often stored for long periods on farms and accidentally composted. In theory there should be an optimum pretreatment regime that will create the best conditions for earthworm growth.

The solids used in section 2.3.4 were composted before being used to support earthworm growth. It is possible, however, to treat separated solids in such a way that composting processes are not initiated, and the solids decompose through the action of mesophilic micro-organisms only.

The two methods of treating waste can be termed thermophilic composting and mesophilic aging. In the former the physical management of the material is such that the heat from microbial metabolic activity is conserved, causing an increase in the temperature to the point where a fresh group of thermophilic micro-organisms operating at approximately 50-70°C continue the process of decomposition until nutritional resources begin to decline, along with metabolic activity, causing a subsequent fall in the temperature and the re-introduction of mesophilic micro-organisms to continue the process of decomposition. In mesophilic decomposition the solids are handled in such a way as to facilitate the loss of heat from the solids and so inhibit the introduction of thermophilic micro-organisms. This can be easily achieved by storing solids in such a way as to increase their surface area to volume ratio and so encourage heat loss.

In this set of experiments two independent methods of solid pre-treatment can be studied rather than comparisons between differences in the same solid over time as occurs in

section 2.3.4.

Based on the results of section 2.3.4 one can develop hypotheses concerning the relative efficacy of the two processes in producing a medium for earthworm growth.

Ecological systems such as decomposing cattle solids obey the basic laws of thermodynamics, and therefore over time tend towards a position of minimum energy and maximum entropy. Maximum earthworm weights can therefore be achieved by introducing them into the solids at the earliest opportunity when the maximum amount of energy and nutrients are available for growth. In practice this can only occur when physico-chemical limits to earthworm survival, such as ammonium ion concentration and ionic conductivity have been reached.

Using the release of metabolic energy as a means of measuring the rate of change of physico-chemical factors within the solids, one would expect composting solids to reach the threshold of earthworm acceptability more quickly than mesophilically aging solids, and therefore provide a suitable environment for earthworm growth more quickly than mesophilic aging. However, by the very nature of the process, composting is less well controlled, and it may be difficult to manage the solids to allow it to be used at the optimum time. The results of section 2.3.4 suggest that the further bio-chemical processes maturing processes within the solid continue, the more diminished is the ability of solids to support earthworm growth.

Given the constraints on experimental design, the hypothesis tested is that mesophilic aging does not advance the decomposition process into the maturing phase to the extent of thermophilic composting, is more easily controlled, and could therefore be expected to support better earthworm growth responses.

The experimental work in this chapter consists of measuring a range of physico-chemical

parameters in solids from several sites as they undergo composting or aging, and then using the material at the end of these processes as a medium for earthworm growth. Growth in the various media will be statistically compared, and also correlated with measured physico-chemical characteristics.

6.1.1) Site Descriptions.

Undigested Solids were collected from Bore Place, NIRD, and Oaklands College as described in section 5.1.1.

6.2) Physico-Chemical Analysis of Aged and Composted Solids.

6.2.1) Materials and Methods; Thermophilic Composting

Solids that were to be used as a substrate for thermophilic composting were placed in specially designed composting bins. They were of a wooden slatted construction, the floor raised 10cm from the ground, and the bins having three enclosed walls and an open front. This allowed access to the solids with forks to allow turning and mixing of the solids. The slatted walls and 2cm diameter holes drilled in the floors of the bins allowed free gas exchange to occur within the piles. The bins were placed within a clear polyethylene tunnel to afford protection from the elements.

Approximately 1 m³ of solids were placed within each bin, and the contents were hand turned approximately weekly to allow even decomposition of the solids, and to discourage the formation of anaerobic pockets within the mass of separated solids. The temperature of the beds was taken daily, at 2cm from the surface, 15cm from the surface and 30 cm from the surface. The results can be seen in graphs 6.2.4.1, 2 and 3.

After 45 days the solids had reached the end of the cooling phase of thermophilic composting, and so were in an acceptable state to be used as a substrate for earthworm growth trials. This was confirmed with earthworm survival tests in a subsample of the solids involved.

6.2.2) Materials and Methods; Mesophilic Aging.

In order to prevent the retention of metabolic heat released by microbial activity, separated solids used in mesophilic aging were kept in modified dustbins, raised above the ground and with many aeration holes. Approximately 0.025m³ of separated solids were kept in each bin and, as with the thermophilically composted solids, turned weekly within the

bins. After 45 days, earthworm survival tests showed that the solids were acceptable to earthworms and so could be used in earthworm growth trials.

6.2.3) Physico-Chemical Analyses of Solids during Pretreatment.

As in section 5.1.4.

6.2.4) Results.

Tables 6.2.4.1 and 2 show the physico-chemical characteristics of aged and composted solids for Bore Place, NIRD and Oaklands College Solids. Graphs 6.2.4.1 to 3 show the temperatures within the solids on composting.

Table 6.2.4.1.

**Physico-chemical Analysis of Thermophilically Composted
Solids from Bore Place, NIRD and Oaklands College.**

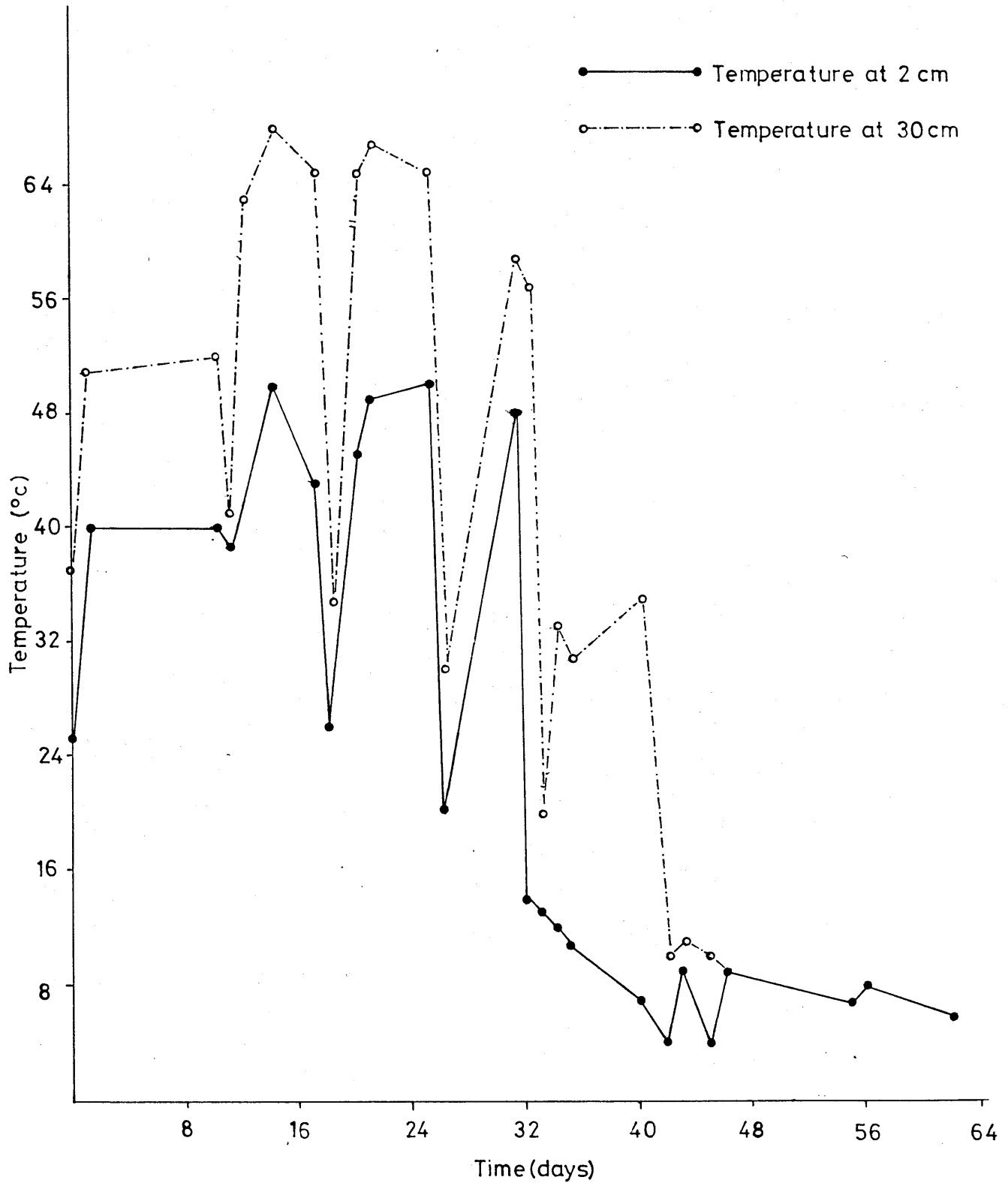
Variable	Site	0 days	5 days	12 days	34 days	45 days
T.S. (%)	Bore	19.4	20.9	20.9	22.7	21.9
	NIRD	16.0	16.0	16.0	15.8	16.2
	Oak	17.1	16.4	17.3	17.0	17.4
V.S.(%)	Bore	85.5	83.1	87.7	78.2	77.5
	NIRD	90.9	79.5	68.9	87.4	74.3
	Oak	82.0	80.1	74.2	77.7	75.7
pH	Bore	9.0	8.3	8.2	8.3	8.5
	NIRD	7.6	8.1	7.9	8.3	8.6
	Oak	8.1	8.0	8.0	8.2	8.3
Ionic Conductivity ($\mu\text{S}/\text{cm}$)	Bore	3370	5630	5920	6930	7180
	NIRD	3100	3130	3260	4110	4230
	Oak	4650	4620	4780	7270	7310
$\text{NH}_3\text{-N}$ (mg/g d wt)	Bore	0.40	0.12	0.07	<0.01	<0.01
	NIRD	0.17	0.11	0.03	0.01	<0.01
	Oak	0.12	0.10	<0.01	<0.01	<0.01
$\text{NH}_3\text{-N}$ and $\text{NH}_4^+\text{-N}$ (mg/g d wt)	Bore	3.07	2.80	2.12	0.01	<0.01
	NIRD	3.97	1.69	0.48	0.26	0.21
	Oak	4.91	0.68	0.07	0.03	<0.01
Total Kj-N (%)	Bore	3.5	3.4	2.9	2.7	2.3
	NIRD	3.3	3.0	2.6	2.0	1.9
	Oak	3.8	3.5	3.0	3.2	2.9
Total Organic Carbon (%)	Bore	73.8	55.9	51.9	49.9	43.9
	NIRD	73.8	62.5	61.2	61.8	59.9
	Oak	71.8	61.8	67.8	55.9	51.9
C/N Ratio	Bore	21.1	16.4	17.9	18.5	19.1
	NIRD	22.4	19.8	18.7	19.7	20.4
	Oak	18.9	17.7	22.6	17.4	17.9

Table 6.2.4.2.

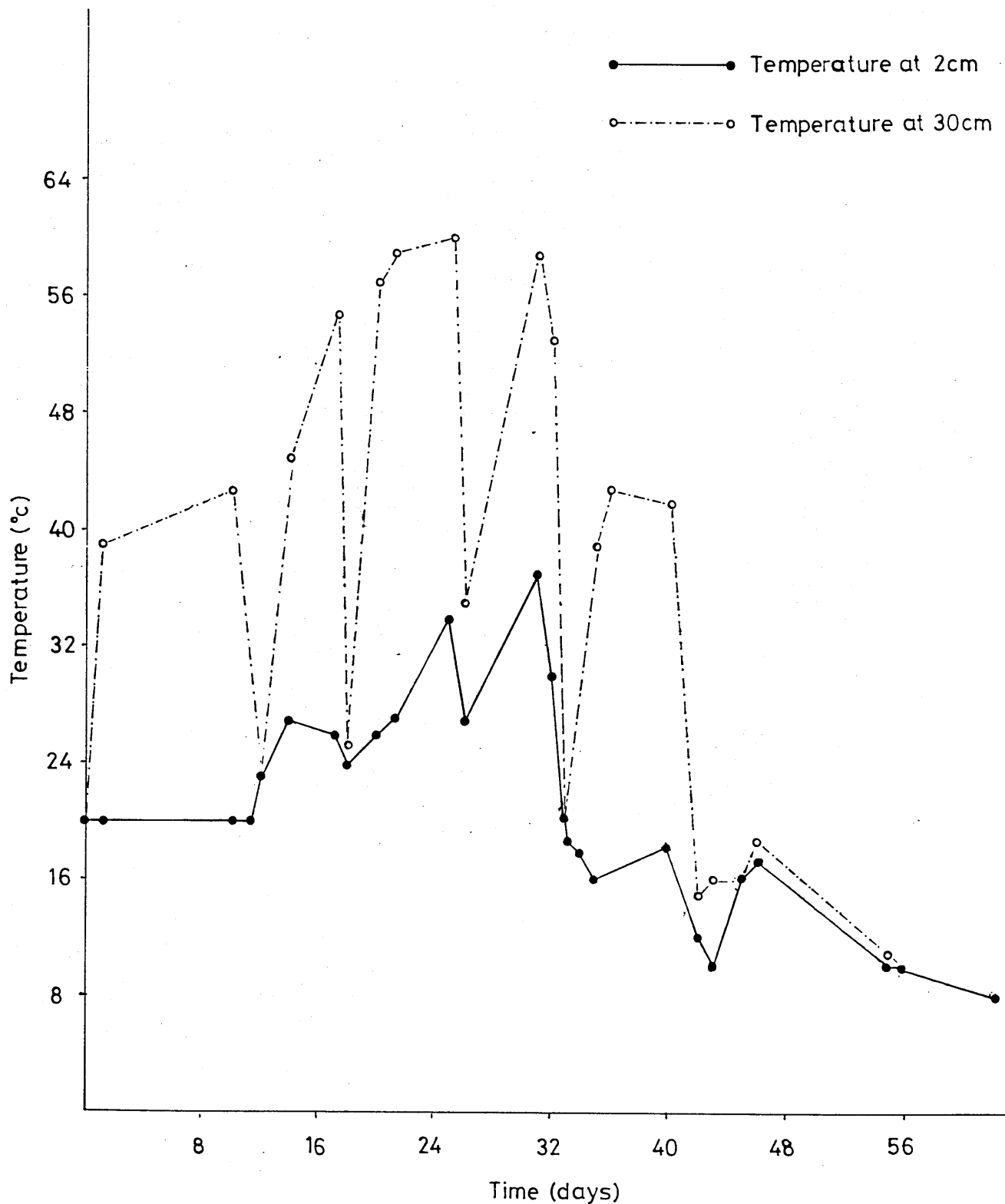
**Physico-Chemical Analysis of Mesophilically Aged
Solids from Bore Place, NIRD and Oaklands College.**

Variable	Site	0 days	5 days	12 days	34 days	45 days
T.S. (%)	Bore	19.4	20.0	19.8	18.2	18.7
	NIRD	16.0	16.8	16.0	15.1	15.7
	Oak	17.1	17.1	17.3	16.6	17.2
V.S.(%)	Bore	85.5	83.9	78.7	79.4	77.8
	NIRD	90.9	83.2	79.5	89.4	87.4
	Oak	82.0	85.6	80.9	84.0	79.8
pH	Bore	9.0	8.8	8.0	8.3	8.9
	NIRD	7.6	8.4	8.8	7.9	8.5
	Oak	8.1	8.5	9.1	8.1	8.6
Ionic Conductivity (μ S/cm)	Bore	3370	3750	4610	6350	6730
	NIRD	3100	3660	2730	4180	4020
	Oak	4620	4590	4570	5210	5630
NH ₃ -N (mg/g d wt)	Bore	0.40	0.28	0.25	<0.01	<0.01
	NIRD	0.17	0.09	0.09	<0.01	<0.01
	Oak	0.12	0.43	0.41	<0.01	<0.01
NH ₃ -N and NH ₄ ⁺ -N (mg/g d wt)	Bore	30.7	2.80	2.12	0.01	<0.01
	NIRD	3.97	3.45	2.19	<0.01	<0.01
	Oak	4.91	4.68	4.05	<0.01	<0.01
Total Kj-N (%)	Bore	3.5	3.6	3.4	3.2	3.1
	NIRD	3.3	3.5	3.2	2.9	3.0
	Oak	3.8	3.5	3.5	3.2	3.3
Total Organic Carbon (%)	Bore	73.8	63.8	51.9	57.2	50.5
	NIRD	73.8	69.2	59.9	57.2	
	Oak	71.8	73.2	67.8	62.5	65.2
C/N Ratio	Bore	21.1	17.7	15.3	17.9	16.3
	NIRD	22.4	19.8	18.7	19.7	20.4
	Oak	18.9	20.9	19.4	19.5	19.7

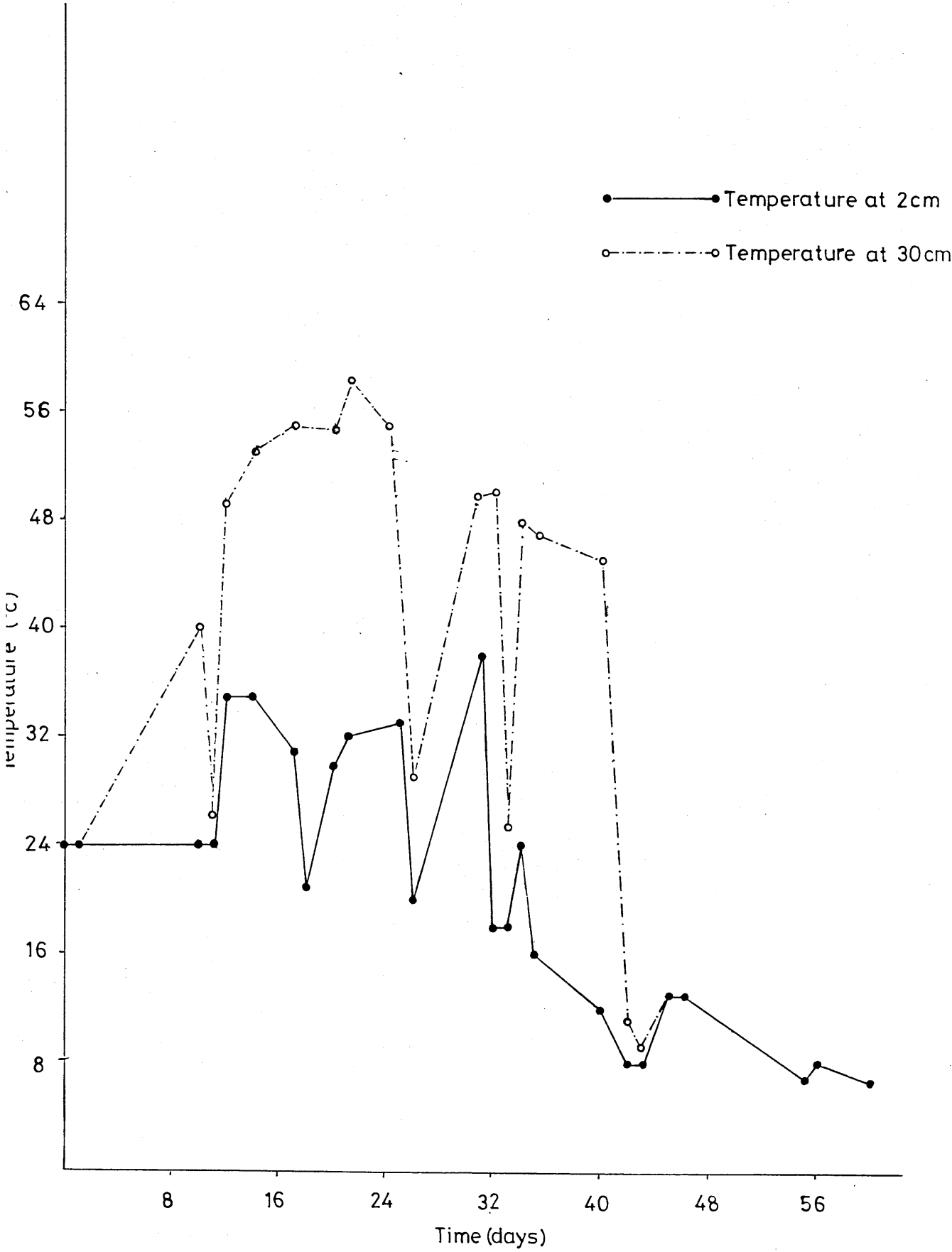
Graph 6.24.1. Temperature of Composting
Bore Place Solids at Two Depths



Graph 6.24.2. Temperature of Composting
NIRD Solids at Two Depths



Graph 6.24.3. Temperature of Composting
Oaklands College Solids at Two Depths



6.2.5) Discussion of Physico-Chemical Analyses with Respect to Aging and Composting.

Bore Place Solids.

For Bore Place material the major differences between the composting and aging process with respect to the physico-chemical measurements are as follows;

There is a reduced loss of moisture from solids undergoing mesophilic aging. It is likely that the lower temperatures recorded on mesophilic aging caused less moisture vapour to be driven off. When turning the composting solids steam could be seen to rise from the newly uncovered solids.

The reduction of NH_3 and NH_4^+ ion concentration from aging and composting solids is similar. The pH of solids undergoing both treatments remains high, favouring volatilisation. Temperature is known to have an important influence on volatilisation. Reviewing the literature, Freney *et al* (1983) show that increasing temperature increases the relative proportion of NH_3 to NH_4^+ , decreases the solubility of NH_3 in water and increases NH_3 diffusion, all of which tend to increase the rate of volatilisation. It could therefore be expected that the higher temperatures of composting would increase NH_3 and NH_4^+ loss through volatilisation. However volatilisation will be increased in aging solids by the following mechanism. To reduce the chance of heat retention the solids undergoing mesophilic aging were spread out to increase the surface area to volume ratio. This would tend to increase the volatilisation of NH_3 and so balance any temperature effects occurring in composting solids. It is important to realise that the values for NH_3 and NH_4^+ ions shown in the tables are just values in points in time and give no indication of the relative fluxes of the NH_3 and NH_4^+ ions in thermophilically composting and mesophilically aging solids.

For example, the results show a greater overall loss of total kjeldahl N for composted solids over the period of measurements. A large fraction of this will have been converted to NH_3 and NH_4^+ ions and either lost through volatilisation and nitrification, but the rate of these transformations will not be measured by single point values of HN_3 and NH_4^+ ion concentration.

Bore Place solids undergoing mesophilic aging exhibits reduced carbon loss which indicates a lower rate of aerobic respiration or reduced decompositional activity, a view supported by the reduced kjeldahl nitrogen loss over the 45 day period. the decreased loss of carbon and nitrogen act to maintain the C/N ratio at a constant level. Gotaas (1956) quotes an optimum C/N ratio for decomposition of 25/1 to 35/1, derived from the fact that during aerobic growth living organisms use 25/35 units of C for every unit of N. A low C/N ratio with an excess of nitrogen increases the rate of the composting process, but leads to a greater loss of N through volatilisation, as observed. These results suggest that the metabolic activity in mesophilically aging solids is less as a result of lower temperatures.

NIRD and Oaklands college solids.

The results and trends observed for Oaklands college are similar to Bore Place solids. It is interesting however that Oaklands solids has a high moisture content yet does not display the signs of anaerobic decomposition that NIRD solids do. This may be due to differences between the bedding material used at the two sites. At NIRD sawdust was employed, whereas at Oaklands college chopped straw was used. The difference in particle size between these two materials will effect the texture of the solids, and especially the pore size within the solids. Oaklands solids may have larger pores and so be able to absorb a similar amount of water to NIRD solids without pores becoming totally filled with water. Indications from the results obtained from the composting of NIRD solids were that a certain proportion of decompositional processes were occurring anaerobically. The reduced loss of carbon as CO_2 and increased nitrogen loss indicates the development

of general anaerobic conditions within NIRD solids, rather than merely anaerobic microsites. In such a situation reduced losses of CO_2 through aerobic respiration, and the increased mineralisation of nitrogen to NH_3 could be expected. The high moisture content of NIRD solids and low temperatures measured during the composting process support this hypothesis. A high moisture content in the solid will block the pores of the material and drastically reduce the rate of oxygen diffusion, allowing anaerobic conditions to develop. In a qualitative examination of the material, malodours associated with anaerobic decomposition were noted. The lower recorded temperatures support this idea. The same material was used in the aging experiment and the physical characteristics which tend towards anaerobicity are still present, although ameliorated by the larger surface area to volume ratio used in mesophilic aging. Unlike the Bore Place solids the differences between the microbial processes occurring in the two situations are not clear cut, as there is no direct measurement of the amount of anaerobic decomposition occurring.

There is little moisture loss under each treatment, and similar rates of ammonia, ammonium and total organic carbon loss. The major difference is in the relative losses of total kjeldahl nitrogen, being much greater in NIRD solids treated to induce composting. If a difference in the microbial metabolic rate is responsible for the observed results, then one would expect increased losses of total organic carbon as well, and this is not the case. Increased TKN loss may therefore be due to N transformations under anaerobic conditions. Microbial denitrification is most likely, but requires a source of NO_3^- ions as a substrate. As discussed earlier nitrification is unlikely to occur in the initial stages of composting, but as the levels of NH_3 drop and become less inhibiting NO_3^- formation may be occurring in aerobic micro-sites within the solids. Because, as argued, a high proportion of NIRD solids may be experiencing anaerobic conditions, there is the possibility of aerobic-anaerobic microsite boundaries occurring, increasing the chances of nitrate denitrification

Another possible route of N loss is through the process known as chemo-denitrification (Clark 1962). In the nitrification process the *Nitrosomonas* group of bacteria converting

NH_4^+ to NO_2^- are more tolerant of high pH and NH_3 concentrations than the Nitrobacter group converting NO_2^- and NO_3^- . Consequently in the sub optimal conditions of 'composting' NIRD solids there may be an accumulation of NO_2^- ions. These can then undergo several chemical transformations to give NO_2 or N_2 as gaseous products which can be lost from the system (Chalk and Smith 1983).

The implications of the physico-chemical changes of composting and aging for earthworm culture are discussed when analysing the results of earthworm growth trials in section 3.1.6.

6.3) The Growth of Earthworms in Composted and Aged solids.

6.3.1) Introduction.

The results of physico-chemical analyses outlined in section 6.2.4 and the interpretation of them in terms of defining the effects of composting and aging on separated cattle solids are only of limited value unless they can be related to some practical process, in this case earthworm culture. The four types of solids from the three sites, having undergone the composting or aging process are ready for use as a medium for earthworm growth and reproduction, where the response of earthworms to the measured physico-chemical characteristics can be observed.

The following experimental pattern is proposed to obtain basic earthworm growth data for individual earthworms with an excess of solids and earthworms competing for nutrition in a crowded situation;

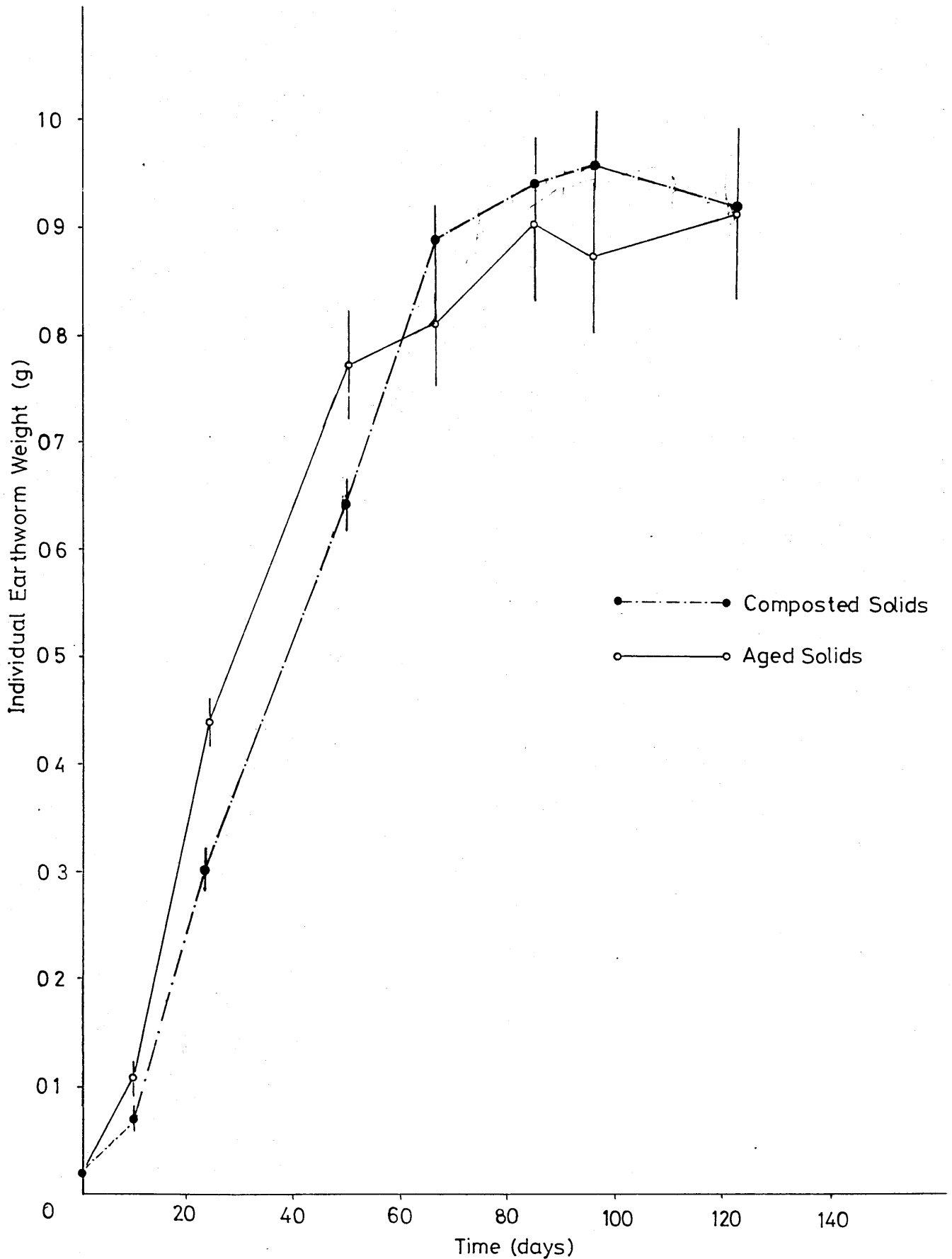
Type of solid	Pre-treatment method	No. earthworms per container
Bore Place Undigested	Composted	1
Bore Place Undigested	Composted	8
Bore Place Undigested	Aged	1
Bore Place Undigested	Aged	8
Bore Place Digested	Composted	1
Bore Place Digested	Composted	8
Bore Place Digested	Aged	1
Bore Place Digested	Aged	8
NIRD undigested	Composted	1
NIRD Undigested	Composted	8
NIRD Undigested	aged	1
NIRD Undigested	aged	8
Oaklands Undigested	Composted	1
Oaklands Undigested	Composted	8
Oaklands Undigested	Aged	1
Oaklands Undigested	aged	8

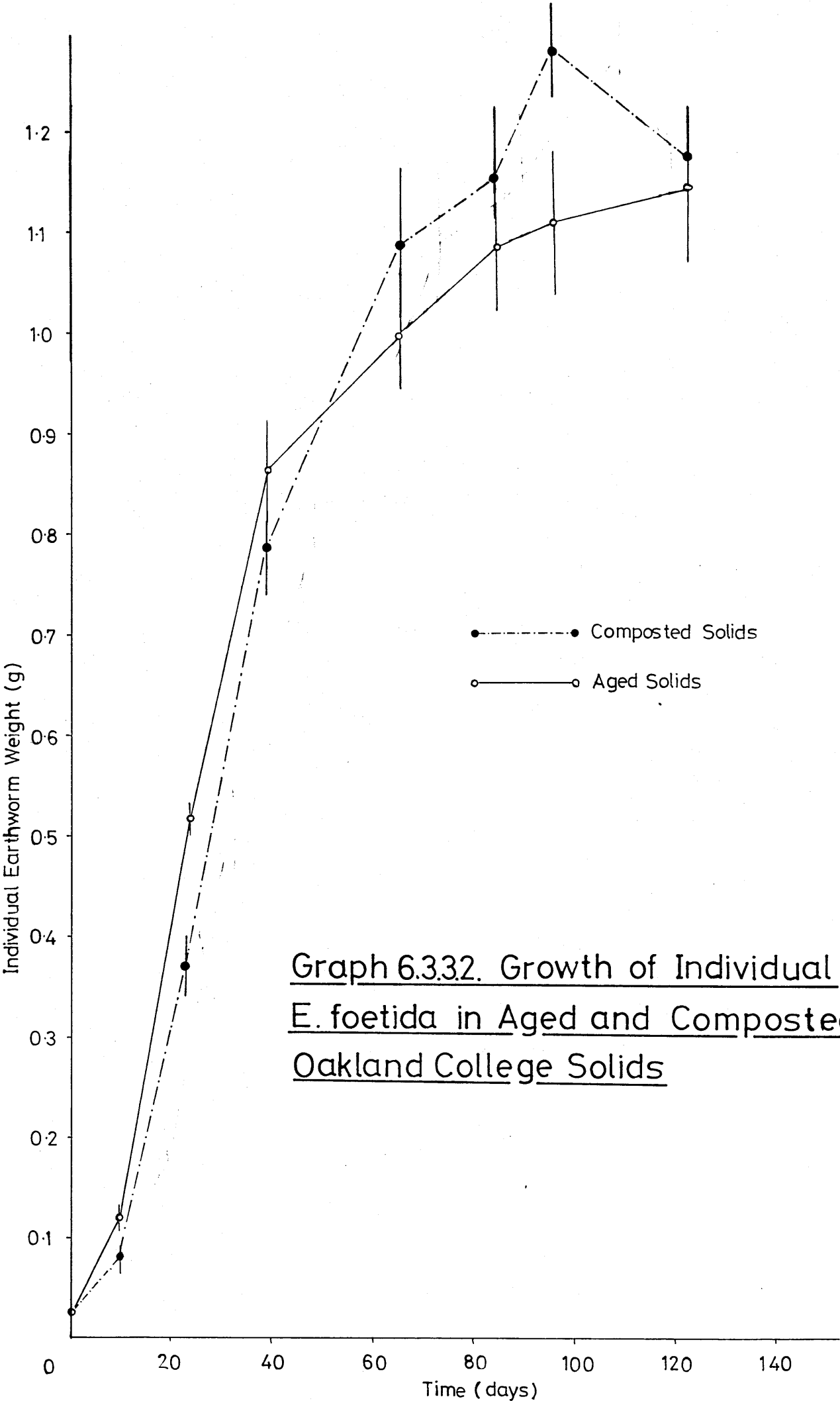
6.3.2) Materials and Methods; As in section 5.2.

6.3.3) Results.

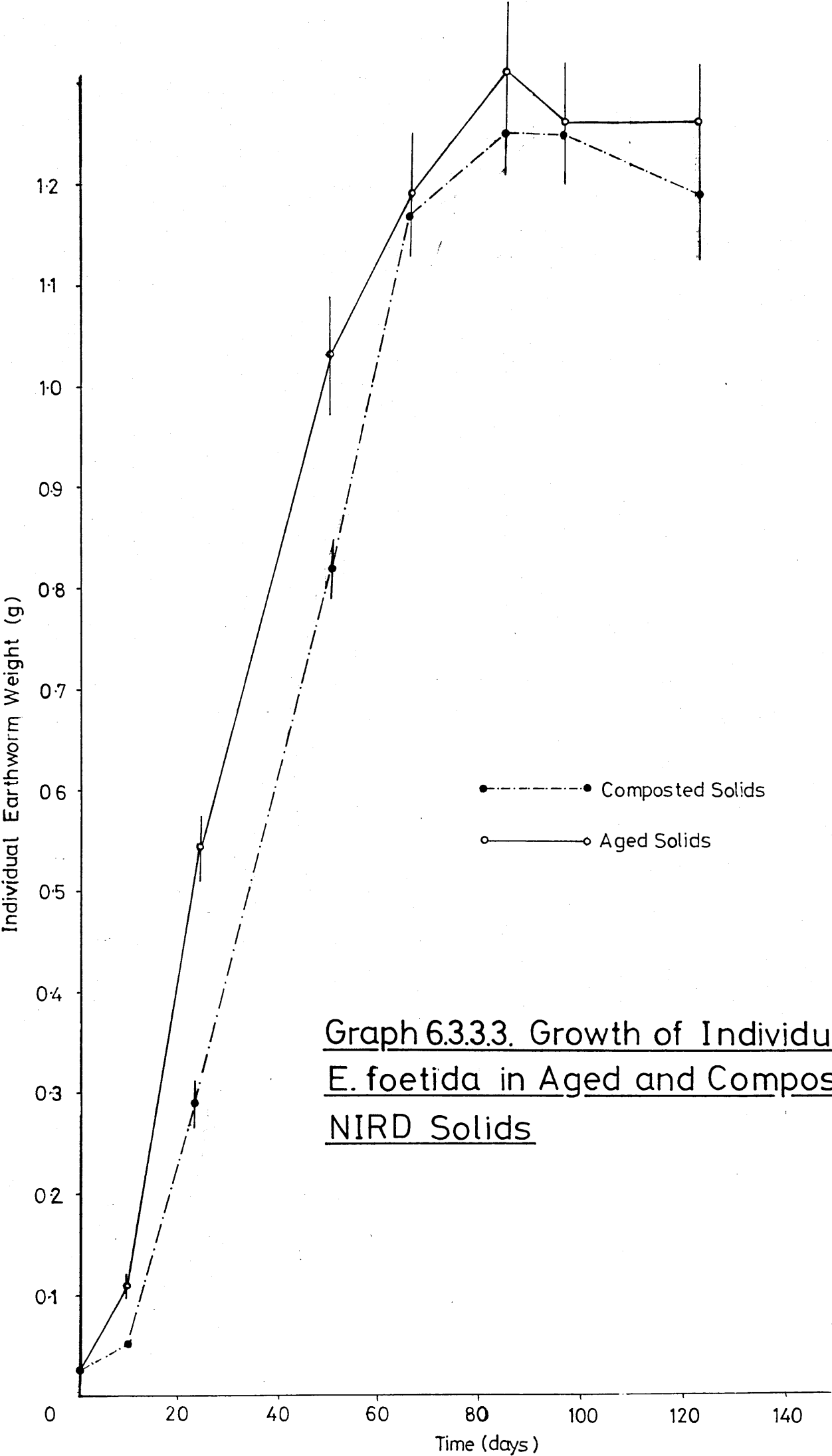
Graphs 6.3.3.1 to 6 show the growth of one and eight earthworms in aged and composted solids from the three sites.

Graph 6.3.3.1. Growth of Individual *E. foetida*
in Aged and Composted Bore Place
Undigested Solids



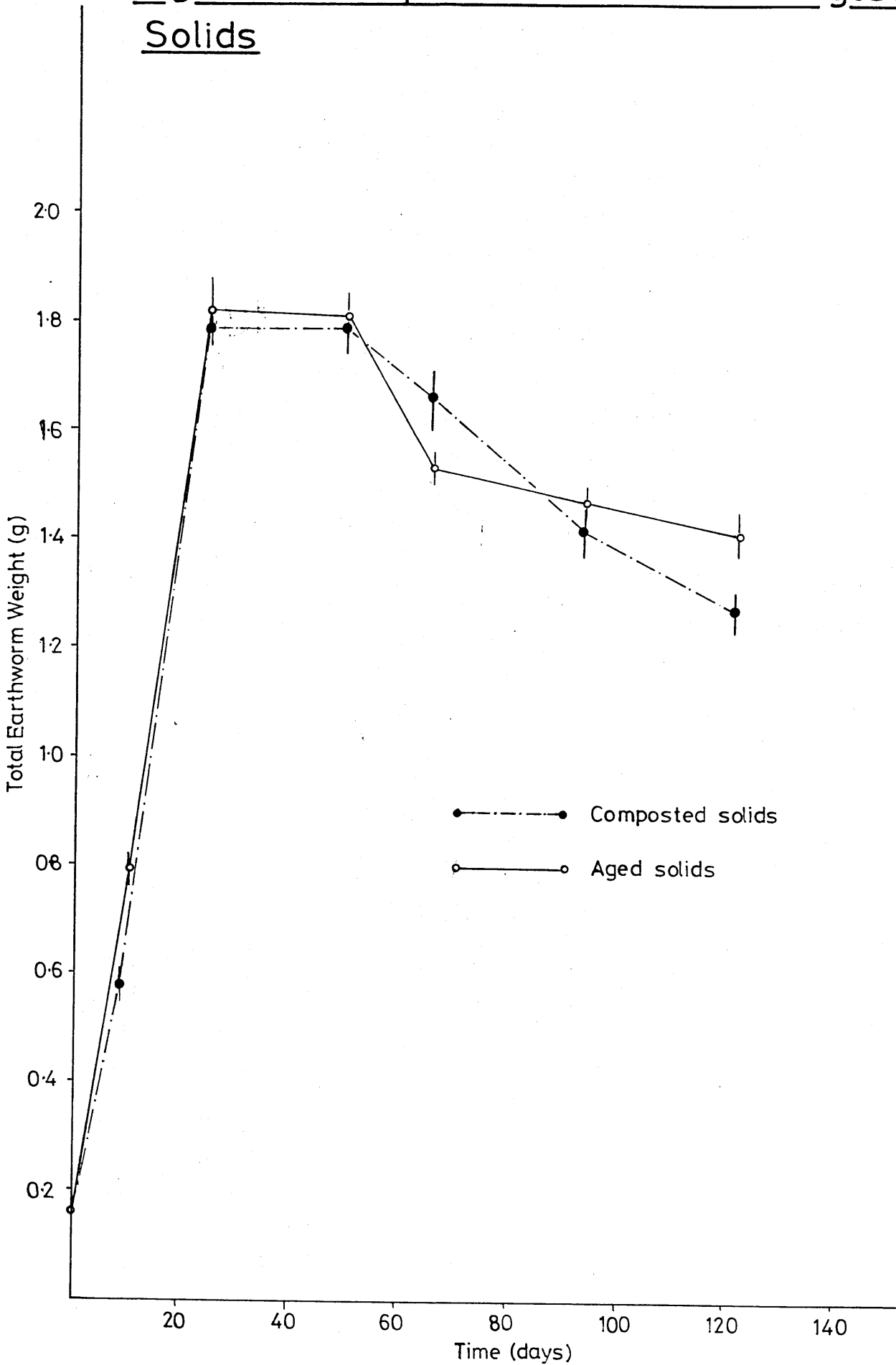


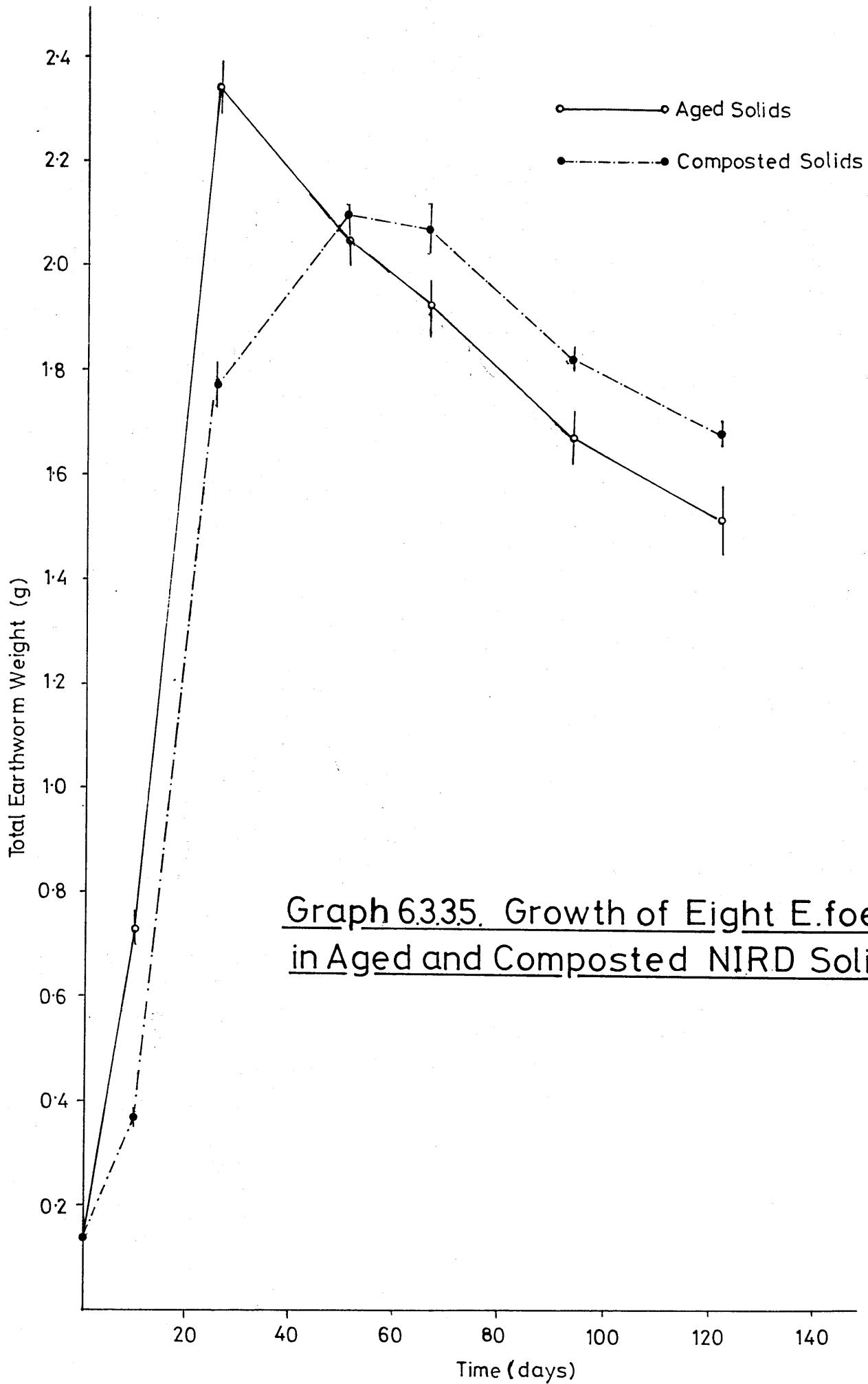
Graph 6.332. Growth of Individual
E. foetida in Aged and Composted
Oakland College Solids



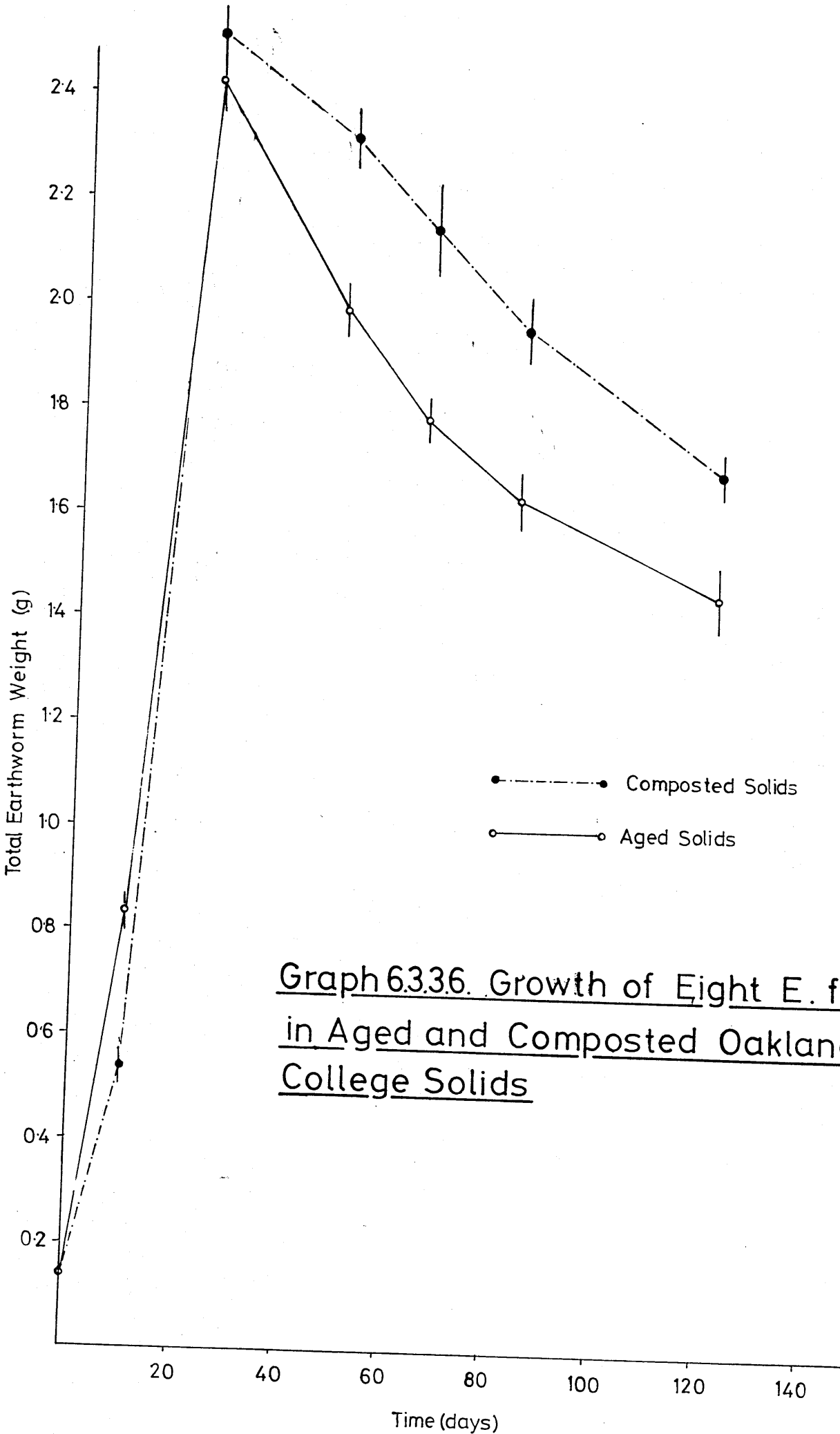
Graph 6.3.3.3. Growth of Individual
E. foetida in Aged and Composted
NIRD Solids

Graph 6.3.34. Growth of Eight *E.foetida* in Aged and Composted Bore Place Undigested Solids





Graph 6.3.35. Growth of Eight *E. foetida* in Aged and Composted NIRD Solids



Graph 6.3.3.6. Growth of Eight *E. foetida* in Aged and Composted Oakland College Solids

6.3.4) Discussion of Results.

The shape of curves presented in the graphs can be defined by three criteria, the initial rate of growth, the peak earthworm weight achieved under the experimental conditions, and the rate at which the earthworm weight declines from the peak value, if this occurs over the course of the experiment. By comparing these three points for the growth curves obtained in the four materials presented, the relative efficacy of composting and aging, between the three sites can be considered. All growth results are related to the physico-chemical analyses.

In comparing the effects of mesophilic aging and thermophilic composting on solids, three responses emerge. For Oaklands college solids and Bore Place undigested solids the aged material has a faster initial growth rate, but the growth of earthworms in composted solids soon equals and peaks at a higher weight than in aged solids. For material from NIRD the aged solids also supports a high initial growth rate, but also goes on to produce the higher maximum earthworm weight of the two processes.

Of these three responses the results obtained from Oaklands solids and Bore Place undigested solids are the most interesting, as it appears that two separate factors are involved. In earlier sections the concept that both environmental and nutritional factors influence earthworm growth has been discussed, and this may be relevant here. No definitive explanation can be given, but the results suggest that the higher ionic conductivity measured in both Bore Place undigested and Oaklands solids initially has an inhibiting effect on the hatchlings used as an inoculum in composting solids. At such an early stage in the experiment where there is such a large amount of solid relative to the earthworm biomass it is unlikely that any nutritional element is limiting. The conductivity measured in the solids was not at a level to cause mortalities, but may have inhibited earthworm growth rate compared to the lower conductivities in aged material. This effect does not persist throughout the course of the experiment, as both individual and eight earthworms

in composted solids finally produce the same biomass

If the ionic conductivity is having the stated effect then there are two possible explanations as to why this effect does not persist throughout the experiment. Firstly, as the *E.foetida* hatchlings mature and gain weight their susceptibility to high ionic conductivities may decrease, although there has been no known research in this area. Secondly, by working the solids, the earthworms may reduce the ionic conductivity to their advantage, possibly through increasing aeration and therefore the volatilisation of ammonia.

Earthworm responses to ionic conductivity cannot fully explain the results obtained, as both the effects described above will be occurring in aged solids as well as composted solids. Differences in maximum weight gain suggests that as the peak in earthworm weight are reached, nutritional factors have become limiting, with composted solids having a greater nutritional value. The results from section 3.1.4 show that both Oaklands and Bore Place undigested composted solids have lower values of total Kj-N and total organic carbon because of losses through increases metabolic activity. However, the results present only a total N or C level with no indication as to what forms the elements are in. To explain the earthworm growth results there must be a much greater percentage of total carbon and nitrogen from composted solids which is available for earthworm nutrition.

The changes that occur on composting have been attributed to increased microbial activity, and it is most likely that a greater proportion of remaining C and N in composted solids has been incorporated into microbial biomass and so available for earthworms. One aspect of the graphs which supports this hypothesis can be seen for eight earthworms cultured in Bore Place undigested solids. Here, the rate of earthworm weight loss is greater in composted solids compared to aged solids after the maximum weight gain has been achieved. Although composted solids have a greater proportion of C and N in earthworm available forms, the absolute amount is less than that of aged solids. Considering that earthworms themselves are considered to stimulate microbial activity (Satchell 1983) one could envisage

a steady incorporation of carbon and nitrogen into microbial biomass, with fibre as the most likely source. This slow release of C and N reserves not expended during the initial aging process during pretreatment will reduce the rate of loss of earthworm biomass after the peak value has occurred.

For solids obtained from NIRD, earthworms cultured in material having undergone mesophilic aging produce a faster initial growth rate, and also the highest weights. This occurs for both individual and eight earthworms. The temperature recorded on composting (see graphs 6.2.4.1 to 3), and the physico-chemical analyses presented on tables 6.2.4.1 and 2 indicate a relatively high level of anaerobic decomposition may have occurred, with consequent increased losses of total nitrogen, and a reduced loss of carbon as CO_2 , the end product of aerobic respiration. The ionic conductivities and pH of the two materials are similar at the end of the pre-treatment process, but composted solids still retain a significant amount of ammonia and ammonium ions which may have an inhibitory effect upon the initial growth rate of the hatchlings. The much reduced total nitrogen levels and possibly a smaller aerobic microbial population could also account for the reduced initial growth rate in composted solids. The lower total biomass in NIRD solids is seen best for eight earthworms where the competition for nutritional resources is greater than for individual earthworms.

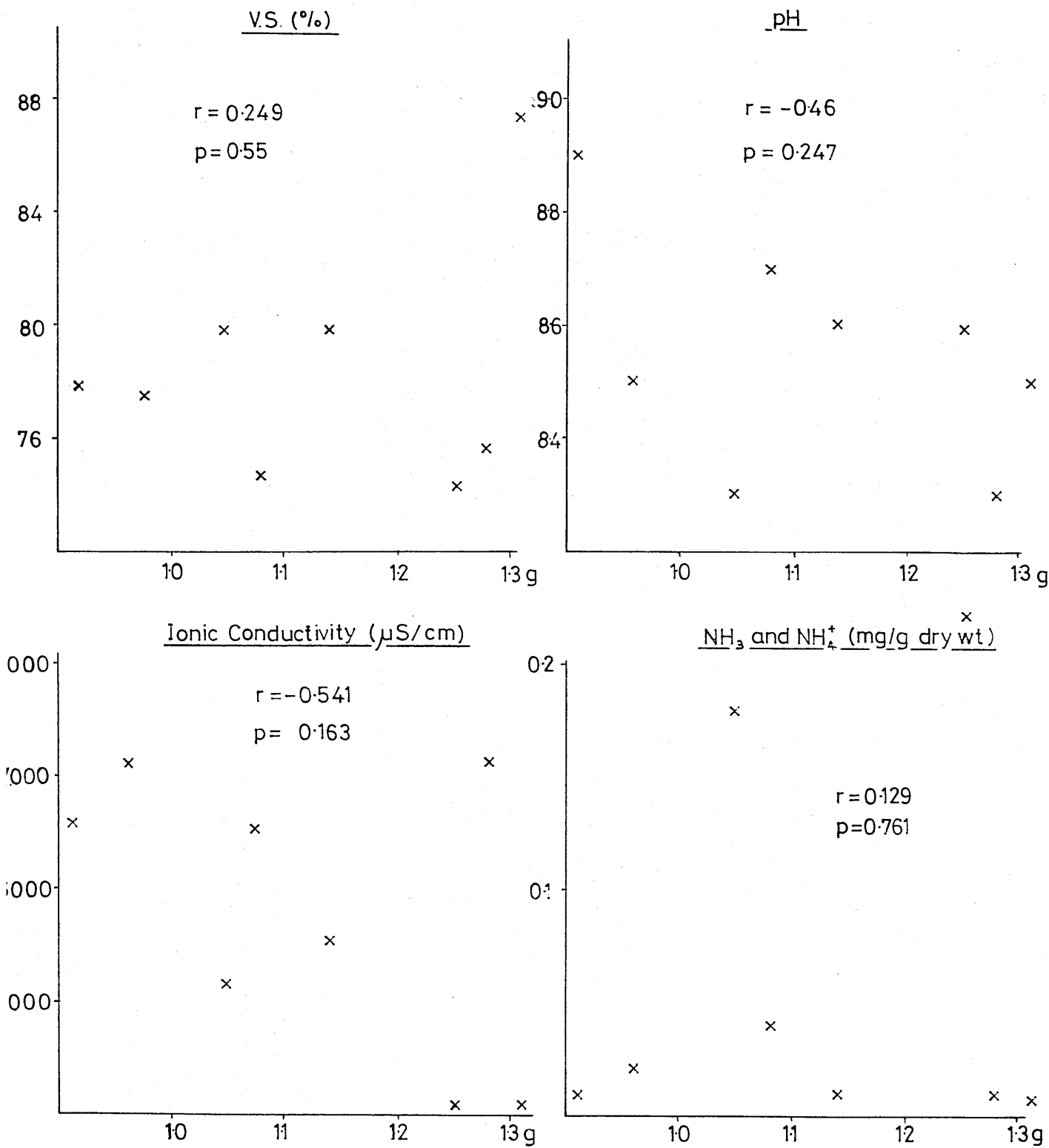
Overall these earthworm growth results show the difficulty in extrapolating information from any one source of agricultural wastes even if it is from the same animal-type and handled in a similar manner. Differences in diet, bedding, the physiological state of the animal, the amount of water that enters the slurry, the type and length of slurry storage and the type and settings of the mechanical separator can be expected to produce different earthworm growth rates between the sites. When pretreated through thermophilic composting or mesophilic aeration, the solids from the three sites cause differences in earthworm growth not just in degree, but also in the pattern of earthworm growth rate, in response to changes in the physico-chemical interactions within solids. The higher

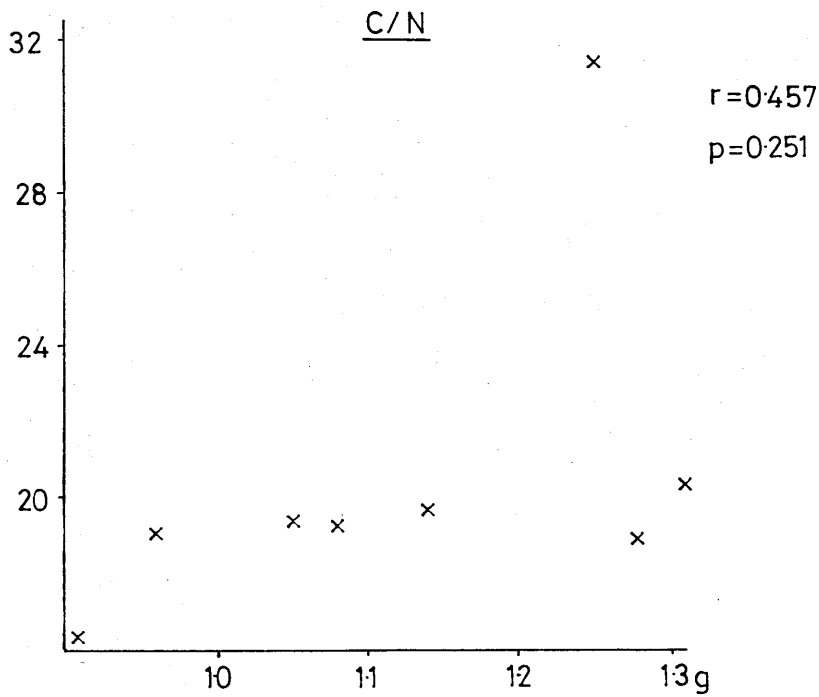
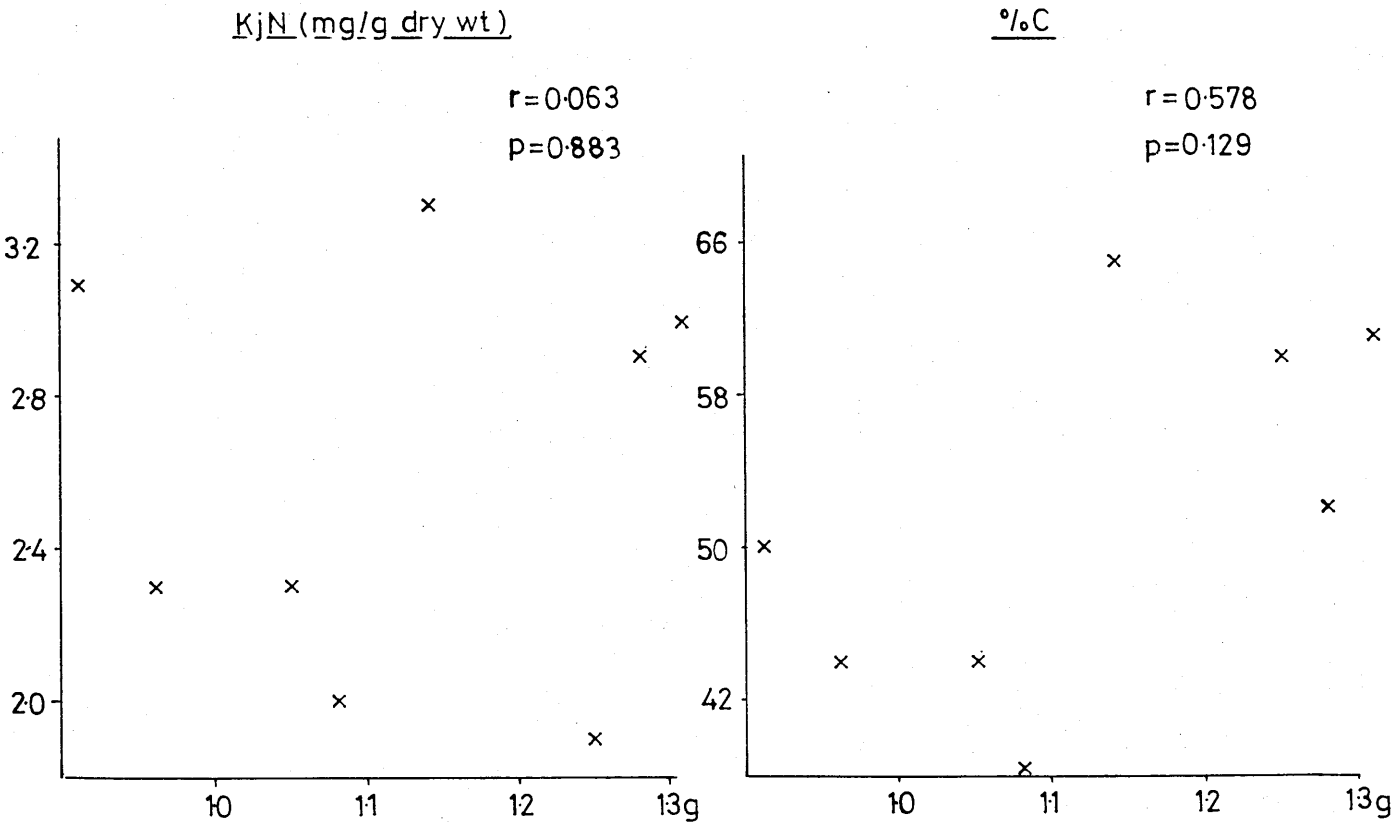
temperatures of thermophilic composting accelerate microbial activity, increasing the rate of C and N loss from the system, but also shifting a greater proportion of C and N into an immediately earthworm available form, most likely microbial biomass. However the higher microbial activity generally creates sub-optimum conditions, with a greater ionic conductivity in Bore Place and Oaklands solids and higher ammonia and ammonium ion concentration in NIRD solids which inhibits the rates of hatchling growth.

6.3.5) Correlation Between Maximum Earthworm Growth and Initial Physico-Chemical factors.

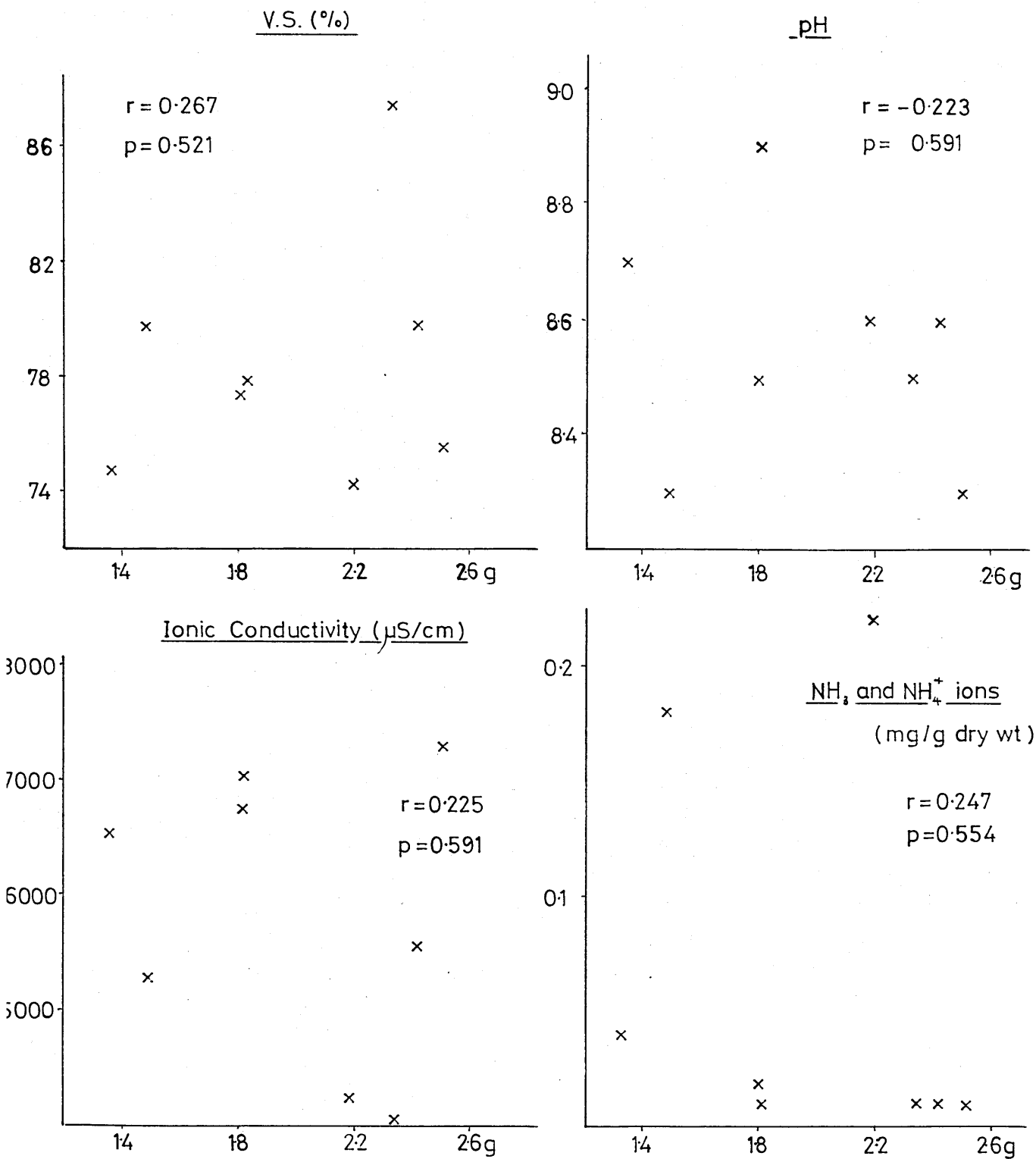
The maximum earthworm weight achieved for one and eight earthworms was plotted against the initial physico-chemical values measured in both aged and composted solids from all sites (including data from chapter seven on digested solids). The correlation coefficient, r , and p , the probability of obtaining such values by chance if there was no correlation between the two variables, was calculated using the SPSSX statistical package. The results can be seen on graphs 6.3.5.1 and 2.

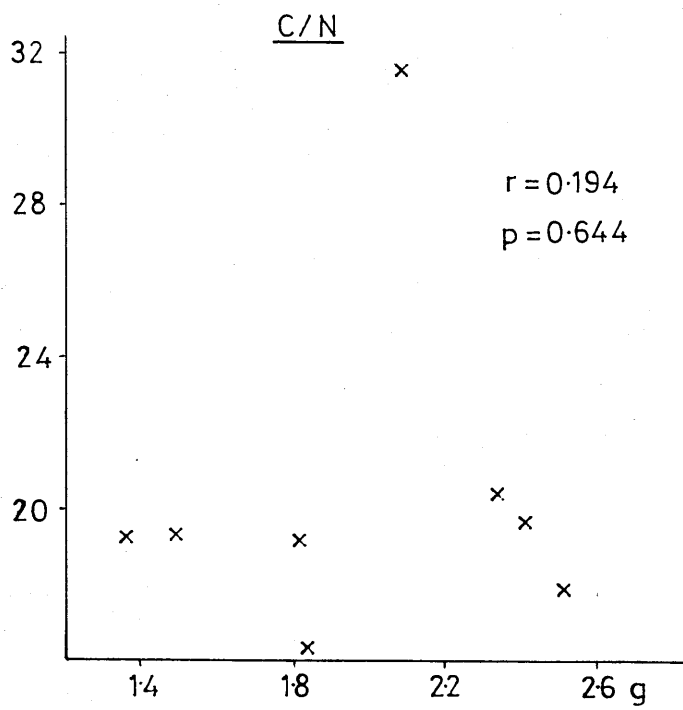
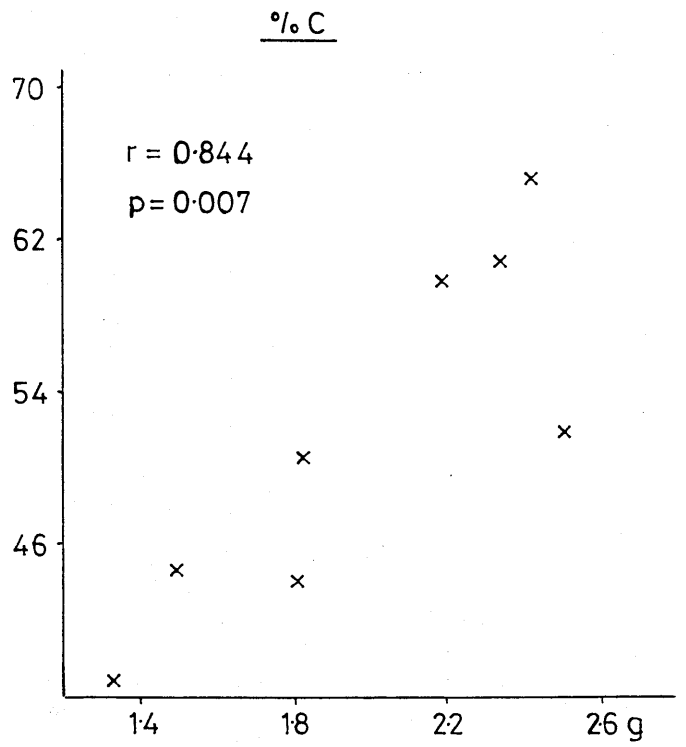
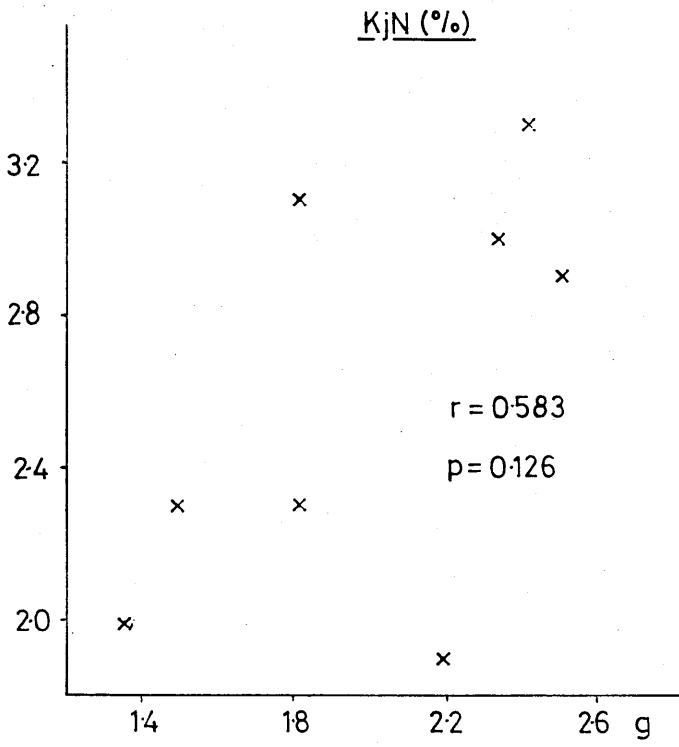
Graph 6.351. Correlation of Maximum Earthworm Weight with Initial Value for Physico-Chemical Characteristics of Several Solids. (one earthworm)





Graph 6.35.2. Correlation of Maximum Earthworm Growth with Initial Values for Physico-Chemical Characteristics of Several Solids. (eight earthworms)





Using the 0.05 or 5% level of significance none of the physico-chemical variables were either positively or negatively statically correlated with the recorded earthworm weights. For one earthworm per container the greatest correlation was with the percentage carbon value, followed by by a negative correlation with ionic conductivity. The least correlation was with NH_3 and NH_4^+ and total kjeldahl nitrogen. For eight earthworms per container the greatest correlation was with percentage carbon, followed by total kjeldahl nitrogen, both positive, with the other variables all having equally low r values.

In arguing that earthworm growth response in seperated solids is affected by a range of inter-related factors, one would therefore not necessarily expect any significant correlation with any one particular factor. However the results obtained are interesting, especially the negative correlation between the maximum weight obtained one earthworm per container and increasing ionic conductivity. This is not replicated in the results of eight earthworms per container.

For eight earthworms per container the greatest correlation is with the two major nutritioal factors measured, total kjeldahl nitrogen and percentage carbon. This is to be expected, because of the greater competition for nutritional resourses at higher earthworm densities and its influence on total earthworm biomass obtained. There is also the possibility that high densities of earthworms can alter the physico-chemical factors of separated solids to their own advantage.

6.3.6) The Relationship of Laboratory Results to the Practical Farming Situation.

The results presented here are difficult to extrapolate to the practical farming situation. As mentioned in the introduction the normal working practice would be to place freshly separated solids directly onto actively working earthworm bed, where by some at present ill defined process the earthworms can move quickly into fresh material and initiate its breakdown, but only if there is a base of acceptable material. This situation is almost

impossible to replicate in the laboratory.

The work on pre-treatment in this section relates most closely the storage of material prior to use in an earthworm system. If on farm storage is required, then the cheapest and most convenient method will be adopted, with little regard to the physico-chemical changes that will be induced. It is most likely that large piles will be created that heat up and start to compost. From the results, the increased metabolic activity will initially create sub-optimum conditions for growth, but will lead to a greater peak after which earthworms will lose weight more quickly. If, as economic reports suggest (see section 1.3.3), the main profit from an earthworm culture system is the worm-worked solids, then the best approach to storage is the management of the solids so that composting is not initiated, and the fast initial growth rate of earthworms can be exploited for the fastest production of worm worked material, and therefore the greatest turnover of solids from a fixed area of worm beds. The results from NIRD solids show that when the solids have a high moisture content, e.g. when exposed to precipitation, the need to avoid stacking the material and therefore increasing the possibility of anaerobic conditions is very important.

Chapter Seven.

The Effects of Anaerobic Digestion on Cattle Solids Used for Earthworm Culture.

7.1) Introduction to Chapter.

Anaerobic digestion has been promoted as an agricultural waste treatment process because of several benefits it affords. Firstly and most obviously is its ability to produce energy in the form of biogas, but more importantly in terms of its use as a waste treatment system are the effects of digestion upon the slurry. These include a reduction in Biological Oxygen Demand, a reduction in total solids, an increase in nutrient mineralization, a reduction in the pathogenicity of the slurry, and a decrease in volatile fatty acid concentration, and therefore fewer offensive odours (Pain *et al* 1985).

With the potential benefits accruing from anaerobic digestion it is desirable to pursue the possibility of linking it with earthworm culture as a combined agricultural waste treatment process, using earthworms to upgrade the value of the separated solid fraction of digester residue with obvious economic benefits (see section 1.1) if it can be achieved.

Such a system can only operate if earthworms can be cultured in treated digested separated cattle solids. In chapters two and three digested solids have been shown to produce no worse results than undigested solids in field earthworm growth trials. In chapter four initial laboratory studies revealed digested solids capable of supporting significantly higher individual earthworm biomasses when compared with undigested solids under similar experimental conditions. The process of anaerobic digestion is therefore compatible with earthworm culture and so justifies further study.

The aim of this chapter is therefore to examine how anaerobic digestion affects the physico-chemical factors of separated digested cattle solids and how this in turn influences earthworm growth. Studying anaerobically digested cattle solids will increase the range of physico-chemical factors encountered in agricultural wastes by earthworms, thus allowing a better model of their responses to be constructed.

The work in this chapter involves experiments on the growth of one and four earthworms in digested solids from two sources, coupled with physico-chemical analyses of the material, and a study of the effects of different pre-treatment regimes upon the physico-chemical factors of digested solids, and thus their earthworm growth characteristics.

The overall hypothesis being tested is that the changes occurring in slurry on anaerobic digestion does not impair the ability of digested solids to support earthworm growth, either by reducing its nutrient content, or changing environmental conditions within the solids to the detriment of earthworms.

7.2) The Effects of Anaerobic Digestion and Mechanical Separation on the Physico-chemical Characteristics of NIRD Slurry.

7.2.1) Introduction.

The only source of digested separated solids described so far in this thesis has been that obtained from the slurry and digester at Bore Place, Edenbridge, Kent. In this chapter digested solids from another source, the Bernard Weitz centre at the National Institute for Research into Dairying (NIRD) are used as a medium for earthworm growth.

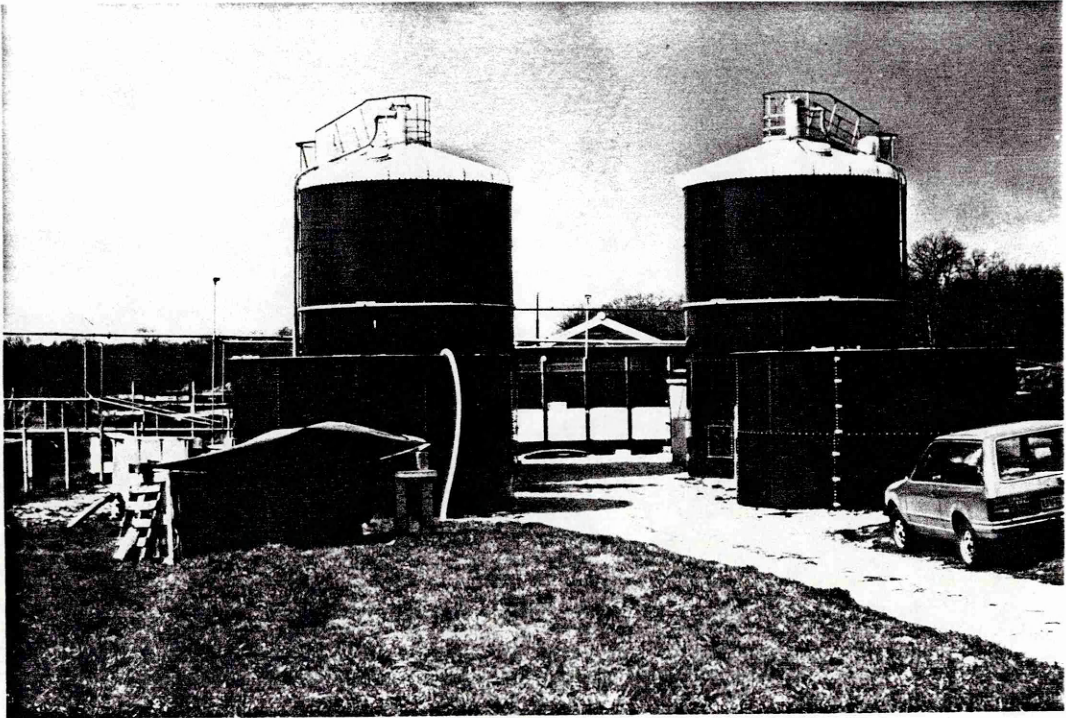
Prior to earthworm growth trials the material is subject to physico-chemical analysis to discern both the effects of different dairy environments and anaerobic digester design and operation on the characteristics of the digested separated solids produced.

7.2.2) Site Description.

For a description of the farm, dairy herd, cattle diet and slurry management facilities see section 5.1.2. The digester in use at NIRD was one of a pair of 125m³ capacity above ground tanks based on a commercial design by Farm Gas ltd, as seen in photograph 7.2.2.1. For more details see Pain *et al* (1984).

Photograph 7.2.2.1.

The Twin Anaerobic Digesters at NIRD.



7.2.3) Materials and Methods.

Samples of undigested and digested dairy cow slurry and the liquid and solid fractions of mechanical separation of the two slurries were collected from NIRD, transported to the Open University and stored in a refrigerator at 4°C.

Physico-chemical analyses of the materials were commenced as soon as possible. These were carried out as in section 5.1.4. Nitrate ions were measured using Devarda's Alloy followed by steam distillation. Potassium and phosphate ion concentrations were calculated by flame photometry and spectrophotometry after dry digestion (MAFF 1983).

7.2.4) Results.

Tables 7.2.4.1 and 2 shows the analysis of the physico-chemical factors of undigested and digested solids and their derivatives.

Table 7.2.4.1.

Undigested Material

Variable	Slurry	Liquid Fraction	Solid Fraction
Total Solids (%)	6.9	4.6	14.7
Volatile Solids (%)	7.1	79.0	91.3
pH	7.7	7.7	8.2
Ionic Conductivity ($\mu\text{S}/\text{cm}$)	11390	14020	8520
NH_3 and $\text{NH}_4^+\text{-N}$ (mg/g dry wt)	31.7	42.4	10.27
$\text{NO}_3^-\text{-N}$ (mg/g dry wt)	trace	trace	trace
Total kjeldahl N (mg/g dry wt)	52.3	87.0	26.3
Organic Carbon (%)	34.5	22.9	39.5
C/N Ratio	6.6	2.6	15.0
Potassium (mg/g dry wt)	65.6	88.5	26.0
Phosphate (mg/dry wt)	24.0	34.8	7.5

Table 7.2.4.2.

Digested Material

Variable	Slurry	Liquid Fraction	Solid Fraction
Total Solids (%)	3.7	3.2	15.0
Volatile Solids (%)	81.6	73.1	94.1
pH	7.7	7.7	8.3
Ionic Conductivity ($\mu\text{S}/\text{cm}$)	13220	13510	5920
NH_3 and $\text{NH}_4^+\text{-N}$ (mg/g dry wt)	37.6	49.7	10.1
$\text{NO}_3^-\text{-N}$ (mg/g dry wt)	trace	trace	trace
Total kjeldahl N (mg/g dry wt)	69.2	88.4	18.9
Organic Carbon (%)	29.3	18.0	59.3
C/N Ratio	4.2	2.7	31.4
Potassium (mg/g dry wt)	71.1	100.3	13.0
Phosphate (mg/dry wt)	46.6	62.5	8.4

7.2.5) Discussion of Results.

The Effects of Digestion Process.

The effects of anaerobic digestion of whole slurry at NIRD is very much in accordance with that observed for the process at Bore Place. Taking the results for undigested and digested slurry these are;

- 1) reduction in total solids by almost one half.
- 2) A reduction in volatile solids.
- 3) A reduction in percentage organic carbon
- 4) No actual loss of nitrogen, but because of carbon loss there is a relative increase in total nitrogen and therefore;
- 5) A reduction in the C/N ratio
- 6) An increase in the mineralization of organic N to NH_3 .
- 7) An increase in the mineralization of organic phosphorus.
- 8) A relative increase in potassium ion concentration because of carbon loss.

The Effects of Slurry Separation.

For a roller press separator as at NIRD and with a slurry at 7% T.S. one would expect 40% to be in the solid fraction and 60% in the liquid fraction on a dry weight basis (Pain *et al* 1978).

The separation process changes the total solids content of the two fractions, producing a free-flowing liquid (T.S. 4.6%) and a stackable non-flowing solid (T.S. 14.7%). The coarse fraction of slurry, which is made up of undegraded fibre from the cattle diet and bedding material is mainly ligno-cellulose and so has a high carbon content. Only finer fibre particles, as defined by the design of the mechanical separator enter the liquid fraction, which therefore has a proportionately lower volatile solids and percentage carbon

value.

A higher proportion of liquid from the original slurry enters the liquid fraction, and thus materials in colloid or solution will be more likely to end up in the liquid fraction. This includes potassium and phosphate ions, as measured, ammonia and ammonium ions in solution and organic nitrogen in colloid, or associated with the microbial biomass which enters the separated liquid fraction. The difference in ionic conductivity between the two fractions is also an indication of this. However it must be remembered that approximately 85% of the solid fraction is water, so a lesser, but still important proportion of ions and colloids are still associated with separated solids.

The effect of the process of anaerobic digestion in reducing the total solids of digested slurry causes the major differences between this and undigested slurry on digestion. Firstly it effects the the proportions of liquid and solid produced. For a digested slurry T.S of 3.7% T.S., 25% of material enters the solid fraction and 75% enters the liquid fraction on a dry weight basis (Pain *et al* 1978).

The major proportion of total solid loss will be from fine rather than coarse particles. Separated digested liquid therefore has a lower T.S. value than undigested separated liquids, although the T.S. of digested solids is similar to undigested solids. The proportion of ammonia and ammonium nitrogen, total nitrogen, potassium and phosphate entering digested liquids and solids is similar. However digested solids have a higher level of organic carbon than undigested solids (53.9% compared with 39.5%). Anaerobic microbial activity on digestion has broken down large carbon molecules and released methane gas as the end product. Therefore the remaining carbon in the digested slurry is likely to be recalcitrant carbon associated with ligno-cellulose complexes which in turn is likely to be found in the large coarse particles of the solid fraction on separation.

The results of the physico-chemical analysis show that there is no fundamental difference

between NIRD undigested and digested solids that would make the material unsuitable for earthworm growth. However anaerobic digestion has produced several differences in the physico-chemical characteristics of digested solids, notably a reduction in the more easily broken down carbon fractions (although this is more likely to affect digested liquids) and an increase in the mineralization of organic materials and thus the ionic conductivity of the material.

In the next section the direct effects of such physico-chemical changes on earthworm growth will be observed and discussed.

7.3) Growth of Earthworms in Digested Solids from Two Sources.

7.3.1) Introduction.

In earlier sections it has been shown that solids derived from digester residue can be used as a medium for earthworm growth, although the only source used so far has been from Bore Place in this section. Earthworm growth trials in undigested and digested solids derived from cattle slurry at Bore Place and NIRD are carried out in order to elucidate the differences digestion brings about to earthworm growth via physico-chemical changes in the slurry and thus the solid.

7.3.2) Materials and Methods.

Undigested and digested solids were collected as in section 7.2.3 and allowed to age for two weeks in the laboratory before becoming acceptable to earthworms. 60g of undigested or digested solids were placed in pots at 85% moisture content and four *E.fetida* added per container. Ten replicates of undigested or digested solids were used, and the containers were incubated at 20⁺1°C, and sampled weekly for earthworm weight as in section 4.2.2.

7.3.3) Results.

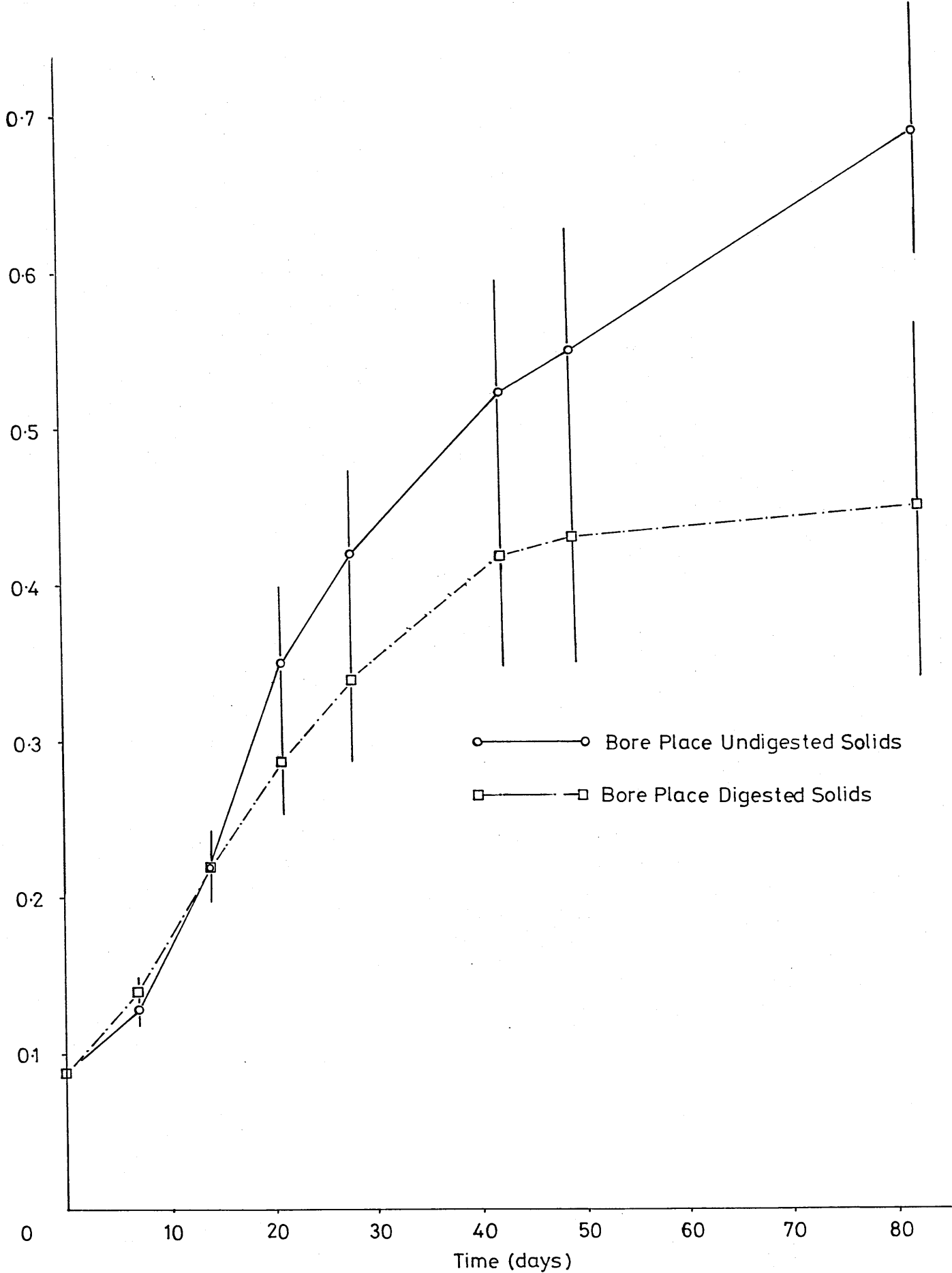
Graphs 7.3.3.1 and 2 shows the growth of one and four earthworms in undigested and digested Bore Place solids respectively. Graphs 7.3.3.3 and 4 shows the growth of one and four earthworms in undigested and digested NIRD solids respectively. Table 7.3.3.1 shows the physico-chemical analysis of freshly collected Bore Place undigested and digested solids. For the values of NIRD material see tables 7.2.4.1 and 2.

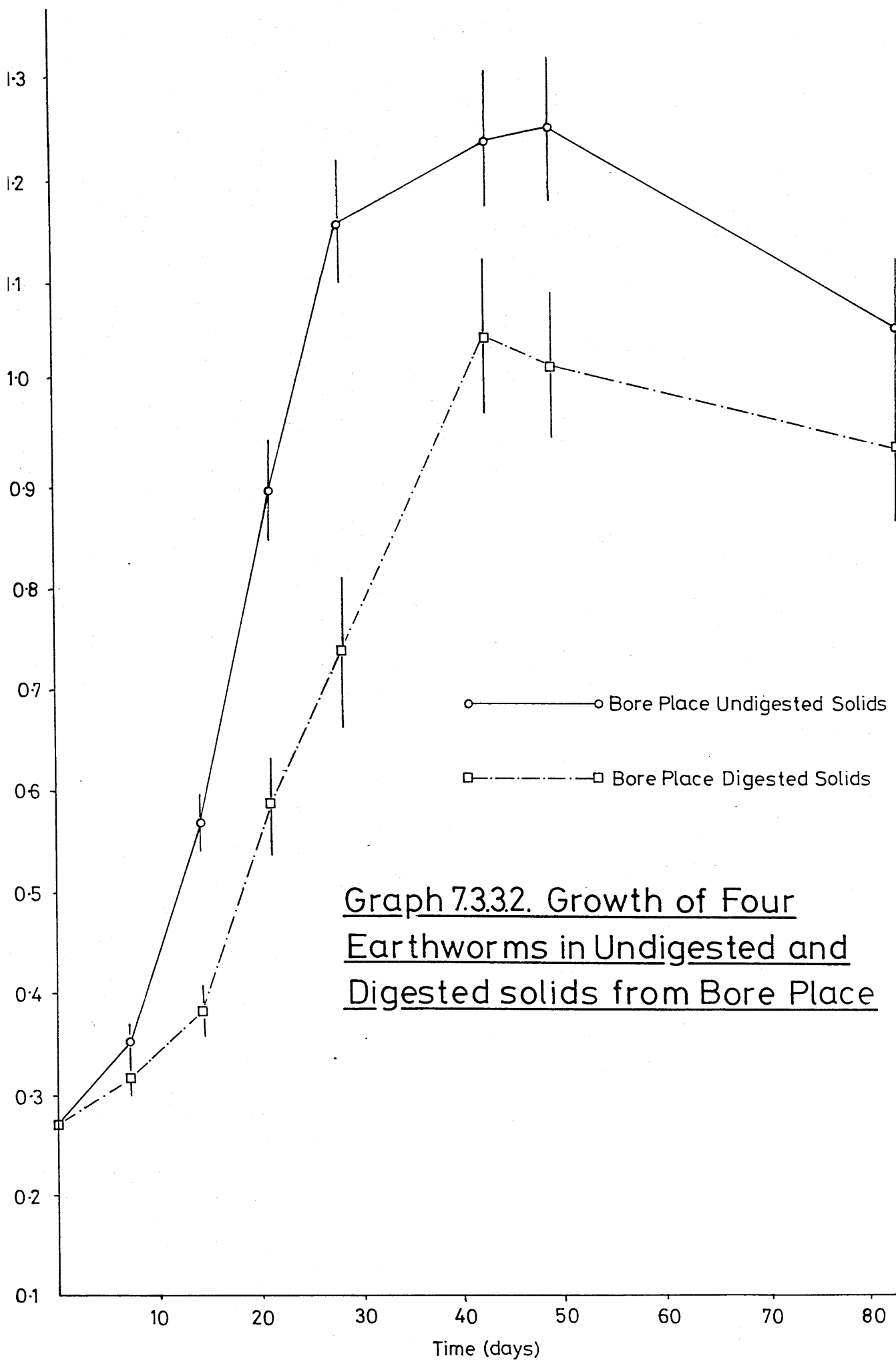
Table 7.3.3.1.

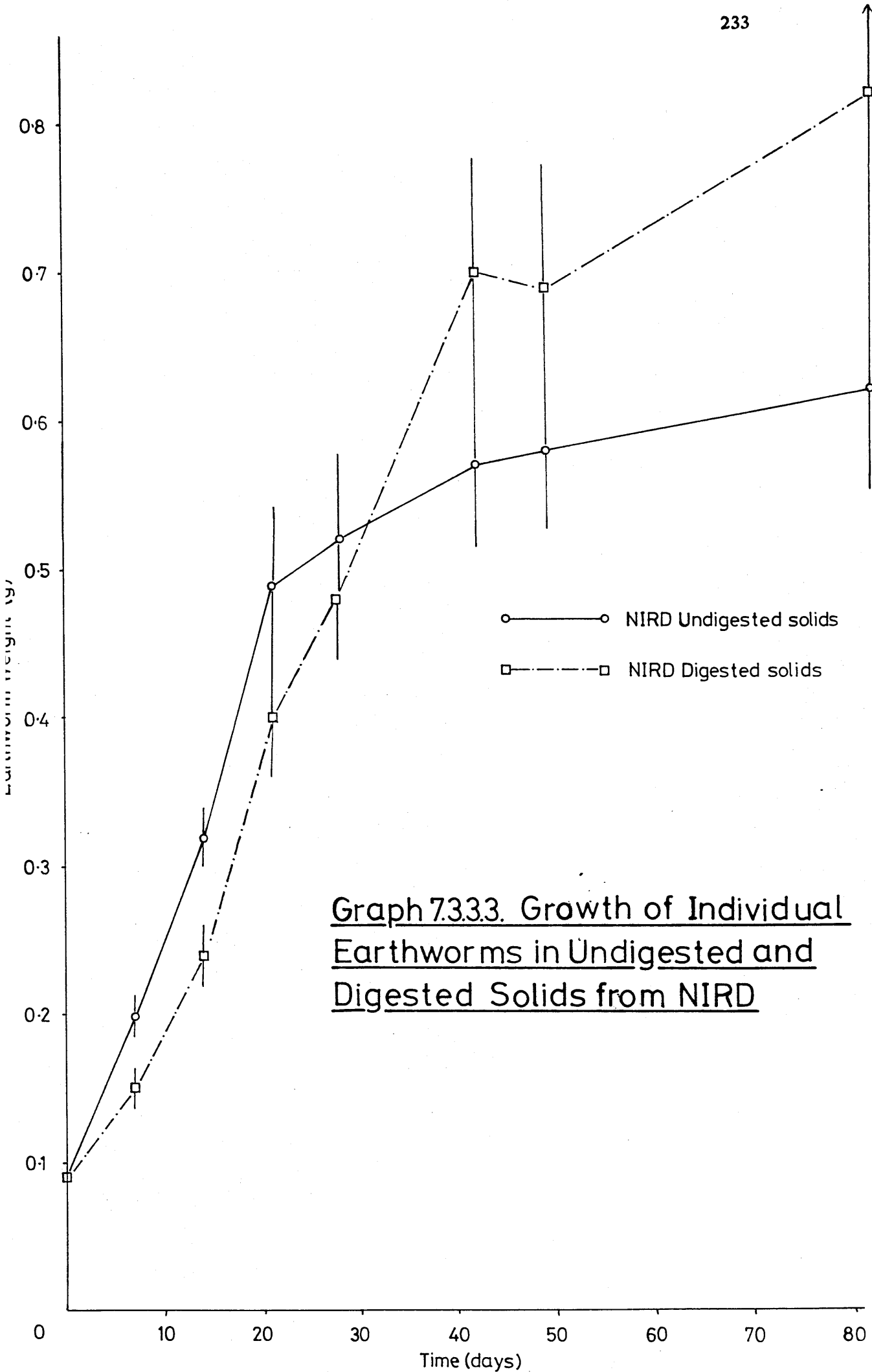
**Physico-chemical Analysis of Freshly Collected Bore Place
Undigested and Digested Solids.**

Variable	Undigested Solids	Digested Solids
T.S. (%)	14.6	15.9
V.S. (%)	88.8	90.3
pH	8.1	8.3
Ionic Conductivity ($\mu\text{S}/\text{cm}$)	8740	11330
NH_3 and $\text{NH}_4^+\text{-N}$ (mg/g dry wt)	7.7	8.7
$\text{NO}_3^-\text{-N}$ (mg/g dry wt)	trace	trace
Total Kj-N (mg/g dry wt)	22.6	23.3
Organic Carbon (%)	43.5	47.9
C/N Ratio	19.2	20.6
P (mg/g dry wt)	12.5	17.1
K (mg/g dry wt)	7.3	11.5

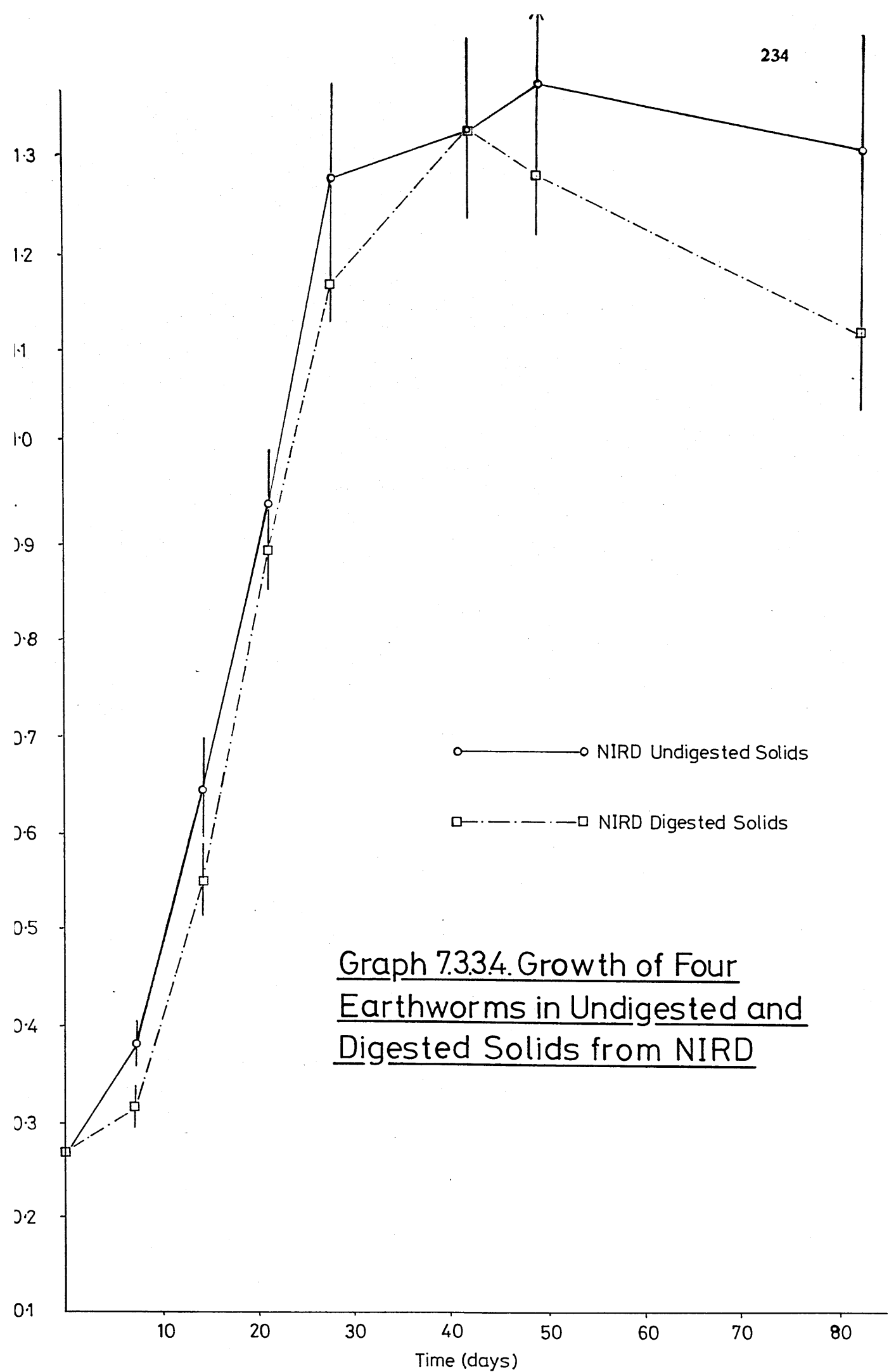
Graph 7.3.31. Growth of Individual Earthworms
in Undigested and Digested Bore Place Solids







Graph 7.3.3.3. Growth of Individual Earthworms in Undigested and Digested Solids from NIRD



7.3.4) Discussion of Results.

Bore Place Solids, One and Four Earthworms.

After fifteen days individual earthworms in undigested solids produced a faster growth rate than digested solids, and after 81 days a higher total biomass that was still increasing (graph 7.3.3.1).

Students t-test was used to compare the mean earthworm weight of the two treatments at each time interval of the experiment. This shows that there is no statistically significant difference between the mean earthworm weight obtained for undigested and digested solids for any of the eight time periods of the experiment at the 5% level of significance.

The reason for the statistical lack of significance between the two treatments, which differs greatly from the impression obtained from the graphical representation of the data, is the large variation in the individual earthworm weights that make up the mean value. This can be seen from the large error bars on the graph.

This indicates that earthworms have produced a wide range of growth responses with the two treatments, generated either through chance, a large variation in earthworm phenotypes present, differences between the solids in each container, or a combination of all three.

A similar response is seen for four earthworms (graph 7.3.3.2). Here earthworms cultured in undigested solids have a higher initial growth and produce a higher final biomass. Using the same statistical criterion as before, undigested solid earthworm weight is significantly higher on day 14 and 42. At the 10% level of significance undigested solids produce significantly higher mean earthworm weights between day 14 and 49.

Such results are in contrast to those in section 4.2. Here individual earthworms cultured in digested solids produced almost double the biomass of equivalents in undigested material, with a slightly smaller difference for eight earthworms.

There was almost a two year gap between the collection of Bore Place material for the two experiments, during which time many changes to cattle diet, slurry handling and the operating of the digester had been carried out as part of general farm management decisions.

In chapter four the response of earthworms to the higher ionic conductivity of undigested solids was seen as the most likely reason for the significant difference between the mean earthworm weights in the two types of solids. From table 7.3.3.1 freshly collected digested solids collected for this experiment had a higher initial ionic conductivity, caused by increased organic N and P mineralization. The increase in conductivity will therefore be in part due to the presence of NH_3 and NH_4^+ ions, also known to be deleterious to earthworms. However digested solids have a higher initial C/N ratio, making conditions more favourable for subsequent decomposition (see section 8.2.4).

Taking the results for one and four earthworms together, although there are physico-chemical differences between digested and undigested solids they have not produced significant differences between the two materials. This may be because the physico-chemical differences are not great enough, or as described above, both positive and negative factors are present, cancelling themselves out with respect to earthworm growth.

NIRD Solids, One and Four Earthworms.

For individual earthworms cultured in NIRD undigested and digested solids an interesting pattern emerges. From a visual inspection of the graph earthworms in undigested solids have a faster initial growth rate for the first 30 days of the experiment, but earthworms

in digested solids produce a higher final biomass at the end of the experiment (graph 7.3.3.3). However, for all but two points the standard error bars of the two growth curves overlap, suggesting no real difference between the growth patterns of earthworms in the two types of solids. This is borne out by the use of Students t-test, which shows a significant difference between undigested and digested solid mean weight at 14 and 21 days in favour of the former, but at no other time, either at the 5 or 10% level of significance. Thus earthworms in undigested solids produced a greater rate of growth initially, but the final earthworm weight was not significantly higher.

For four earthworms growing in NIRD solids there is no difference in the initial growth rate of earthworms in the two materials, but after the peak earthworm weight has been achieved the rate of loss of earthworm weight in digested solids is greater than for undigested solids. Again, these trends are visible on graph 7.3.3.4 but there is no statistical significance between any mean earthworm weight at any time period, either at the 5 or 10% level of significance.

The decline in weight after four earthworms had reached a peak suggests that the earthworm available nutrients within the material were consumed, leading to a subsequent decline in weight. The more rapid decline in digested solids indicates that this material has a lower nutrient content, in keeping with the observed physico-chemical changes on anaerobic digestion and the subsequent effect this has on the mechanical separation process. Such changes are not great enough to produce significantly different results between earthworms growing in the two materials.

7.3.5 General Discussion.

The overall results of earthworm growth patterns in digested solids from both sites indicates that no deleterious effects can be related to the process of anaerobic digestion and the separated solids produced therefrom. This is in agreement with results from

field scale trials in chapters two and three, and initial laboratory trials in chapter four. Although there were differences between earthworm growth responses to the two different separated solid types, the within treatment variation was large enough to statistically cancel out any between treatment variation that may have been present.

These results therefore support the hypothesis that the two processes of anaerobic digestion and earthworm culture could be theoretically linked as part of an integrated system of farm animal slurry management, utilising the advantages of both processes, the control of pollution, ease of handling wastes and upgrading their value.

7.4) The Effects of Anaerobic Digestion Followed by Aging or Composting on Bore Place Solids, and its Ability to Support Earthworm Growth.

7.4.1) Introduction.

This section continues work initiated in chapter six, but as the results deal directly with the changes brought about by anaerobic digestion the section is more relevant in this chapter. The work sets out to test the hypothesis stated in section 7.1.

Undigested and digested solids are compared physico-chemically over time and through the growth of earthworms in the two materials. Digested solids are also pretreated by aging and composting and the effect this has on physico-chemical factors and earthworm growth within the material.

7.4.2) Materials and Methods.

Freshly separated undigested and digested solids from Bore Place were collected and transported to the Open University. During the period that samples were taken the digester at Bore Place was running on a 40 day retention time. Samples were immediately taken for analysis and then the material was pre-treated in the following manner using the protocol described in section 6.1.3.

The following setups were used to allow comparison between digested and undigested solids having undergone two treatments. Discussion of the changes brought about by aging and composting in undigested solids has been covered in section 6.3.4.

Undigested Solids- Aging

Digested Solids- Aging

Digested Solids- Composting

Physico-chemical analysis was carried out as in section 5.1.4, and one and eight earthworms were cultured in 60g wet weight of the two types of solid as in section 5.1.5.

7.4.3) Results.

Graphs 7.4.3.1 and 2 show the growth of one and eight earthworms in Bore Place undigested and digested solids. Graphs 7.4.3.3 and 4 shows the growth of one and eight earthworms in aged and composted Bore Place solids. Graph 7.4.3.5 shows the temperatures recorded in digested solids as it composts. Table 7.4.3.1 show changes in the physico-chemical characteristics over time for aging undigested solids, aging digested solids and composting digested solids respectively.

Table 7.4.3.1.

**Physico-Chemical Analysis of Bore Place Solids
Having Undergone Various Treatments**

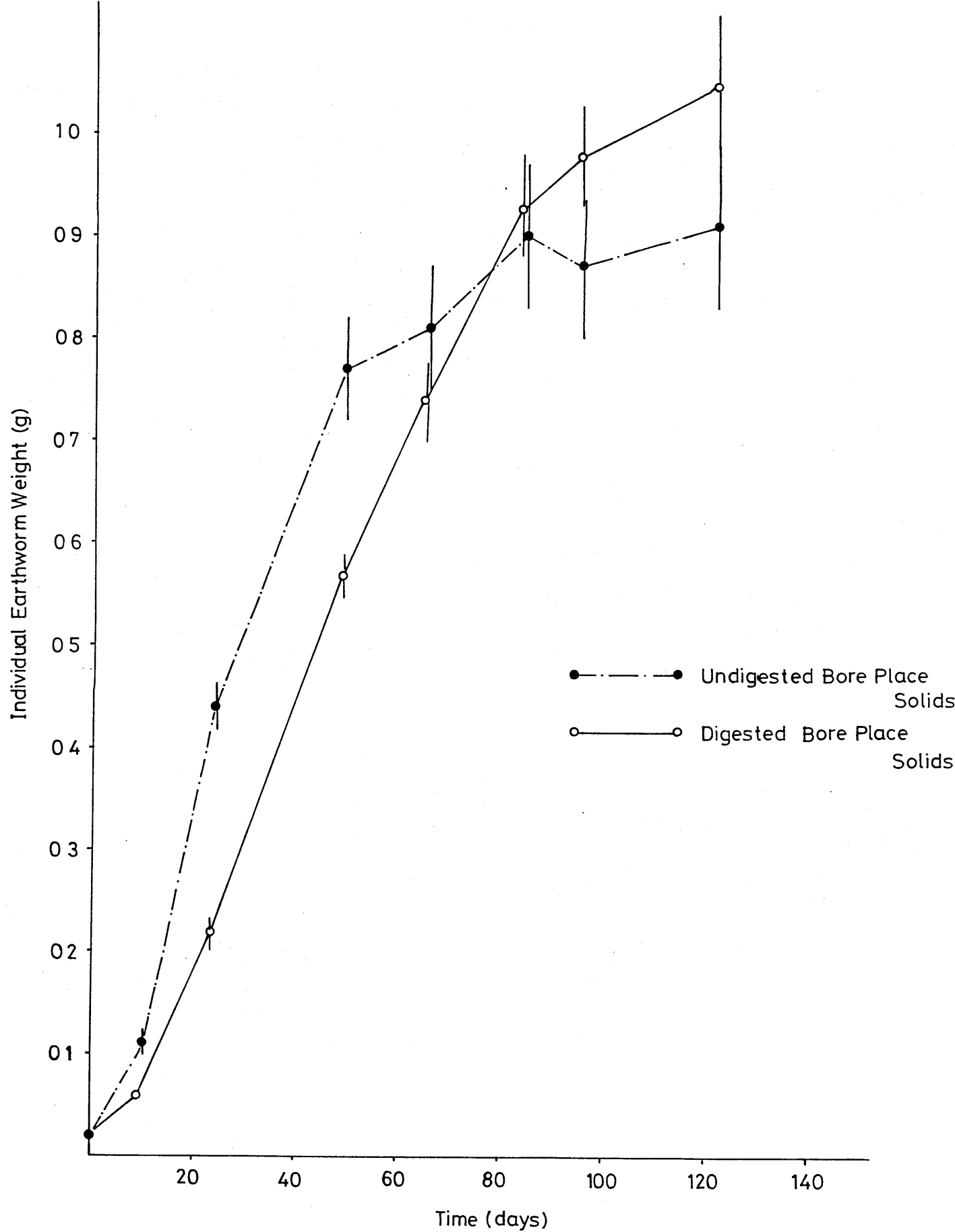
Variable	Treatment	0 days	5 days	12 days	34 days	45 days
T.S. (%)	BPU Age	19.4	20.0	19.8	18.2	18.7
	BPD Age	18.1	18.3	18.6	18.6	19.1
	BPD Com	18.1	19.3	24.4	20.5	21.6
V.S.(%)	BPU Age	85.5	83.9	78.7	79.4	77.8
	BPD Age	85.3	84.7	85.6	82.6	79.8
	BPD Com	85.3	84.9	82.2	82.8	74.7
pH	BPU Age	9.0	8.8	8.0	8.3	8.9
	BPD Age	9.0	8.7	8.9	7.8	8.3
	BPD Com	9.0	7.8	7.6	8.2	8.7
Ionic Conductivity (μ S/cm)	BPU Age	3370	3750	4610	6350	6730
	BPD Age	4250	4380	4520	5560	5350
	BPD Com	4250	4810	4530	6220	6540
NH ₃ -N (mg/g d wt)	BPU Age	0.40	0.28	0.25	<0.01	<0.01
	BPD Age	1.12	0.99	0.94	0.01	<0.01
	BPD Com	1.12	0.01	0.01	0.01	<0.01
NH ₃ -N and NH ₄ ⁺ -N (mg/g d wt)	BPU Age	3.07	2.80	2.12	0.01	<0.01
	BPD Age	9.48	8.20	7.15	0.75	0.18
	BPD Com	9.48	0.78	0.19	0.05	0.04
Total Kj-N (%)	BPU Age	3.5	3.6	3.4	3.2	3.1
	BPD Age	2.45	2.50	2.38	2.36	2.30
	BPD Com	2.45	2.40	2.32	2.18	2.00
Total Organic Carbon (%)	BPU Age	73.8	63.8	51.9	57.2	50.5
	BPD Age	47.9	47.2	41.9	42.6	44.6
	BPD Com	47.9	39.9	35.9	39.9	38.6
C/N Ratio	BPU Age	21.1	17.7	15.3	17.9	16.3
	BPD Age	19.55	18.9	17.6	18.1	19.4
	BPD Com	19.6	16.6	15.5	18.3	19.3

BPU Age = Mesophilically Aged Bore Place Undigested Solids

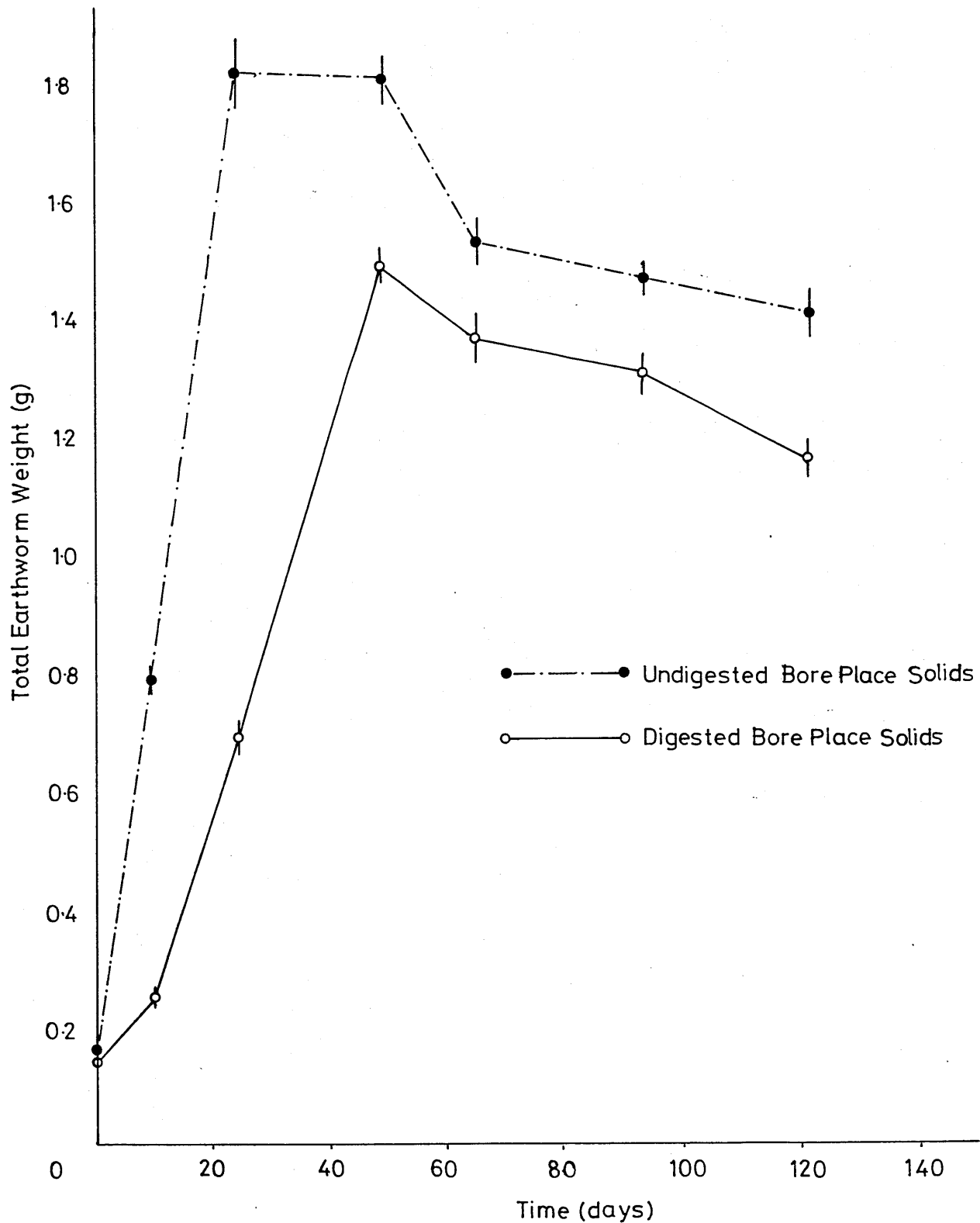
BPD Age = Mesophilically Aged Bore Place Digested Solids

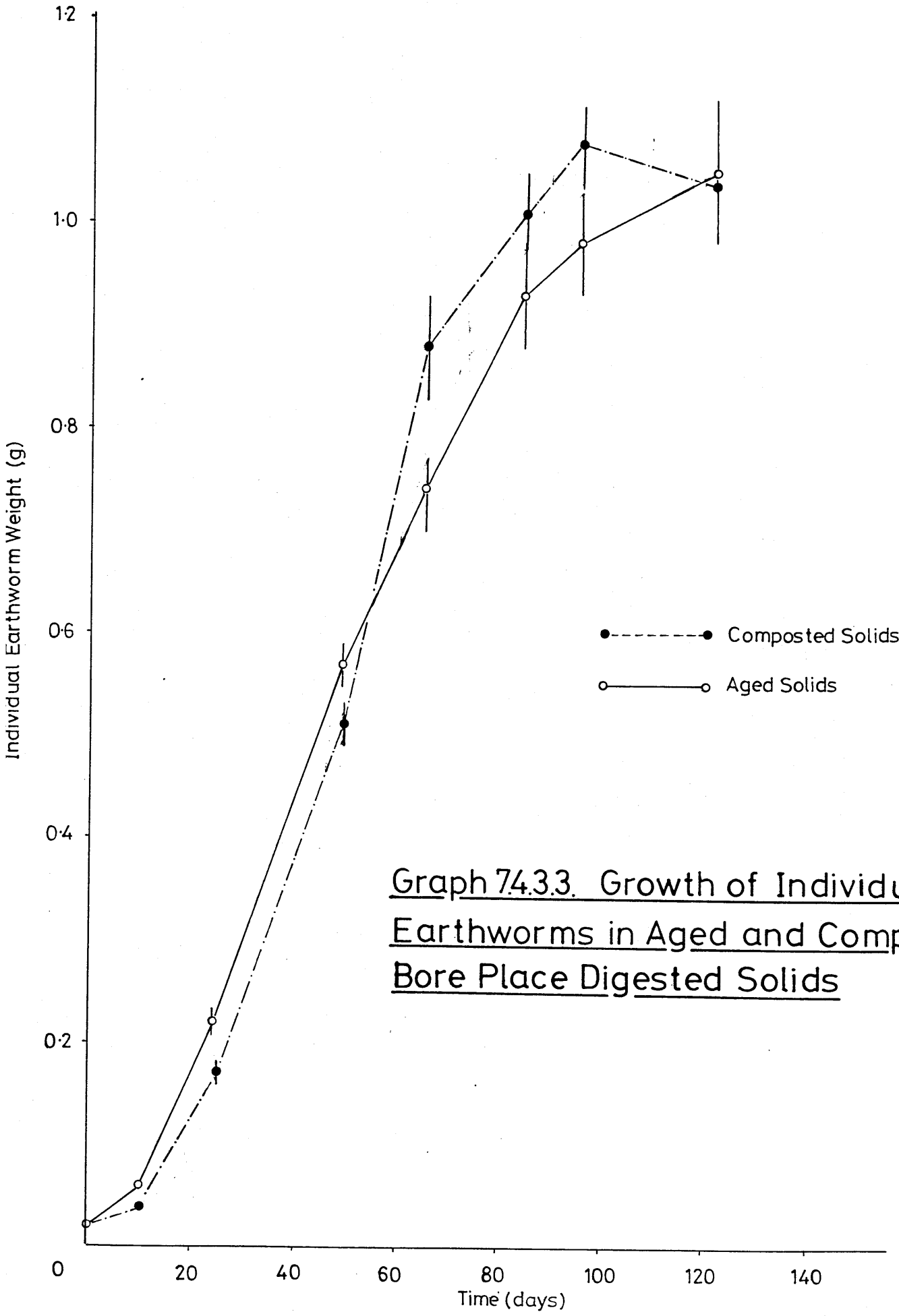
BPD Com = Thermophilically Composted Bore Place Digested Solids

Graph 74.3.1. Growth of Individual Earthworms
in Undigested and Digested Bore Place Solids



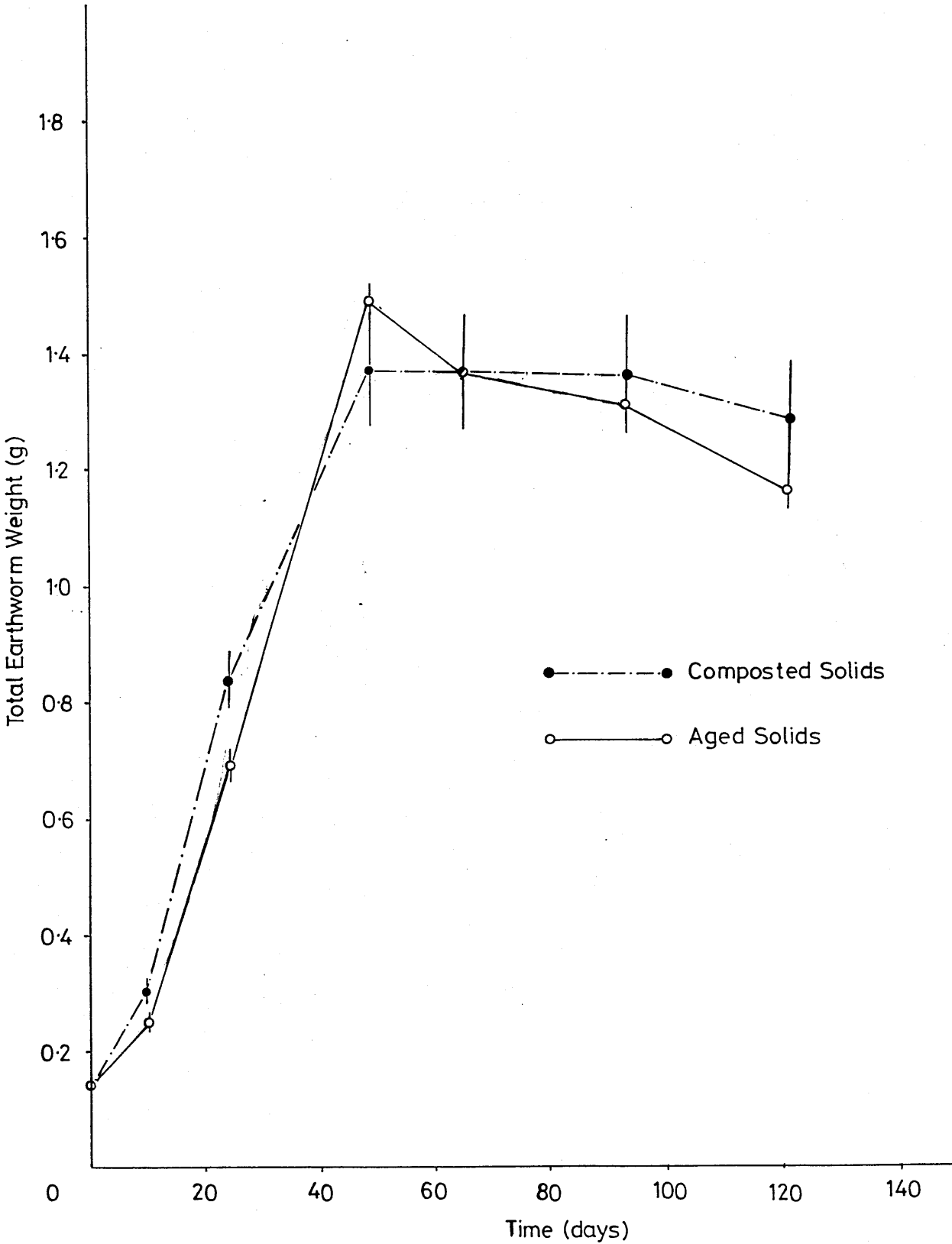
Graph 74.32. Growth of Eight Earthworms in Undigested and Digested Bore Place Solids



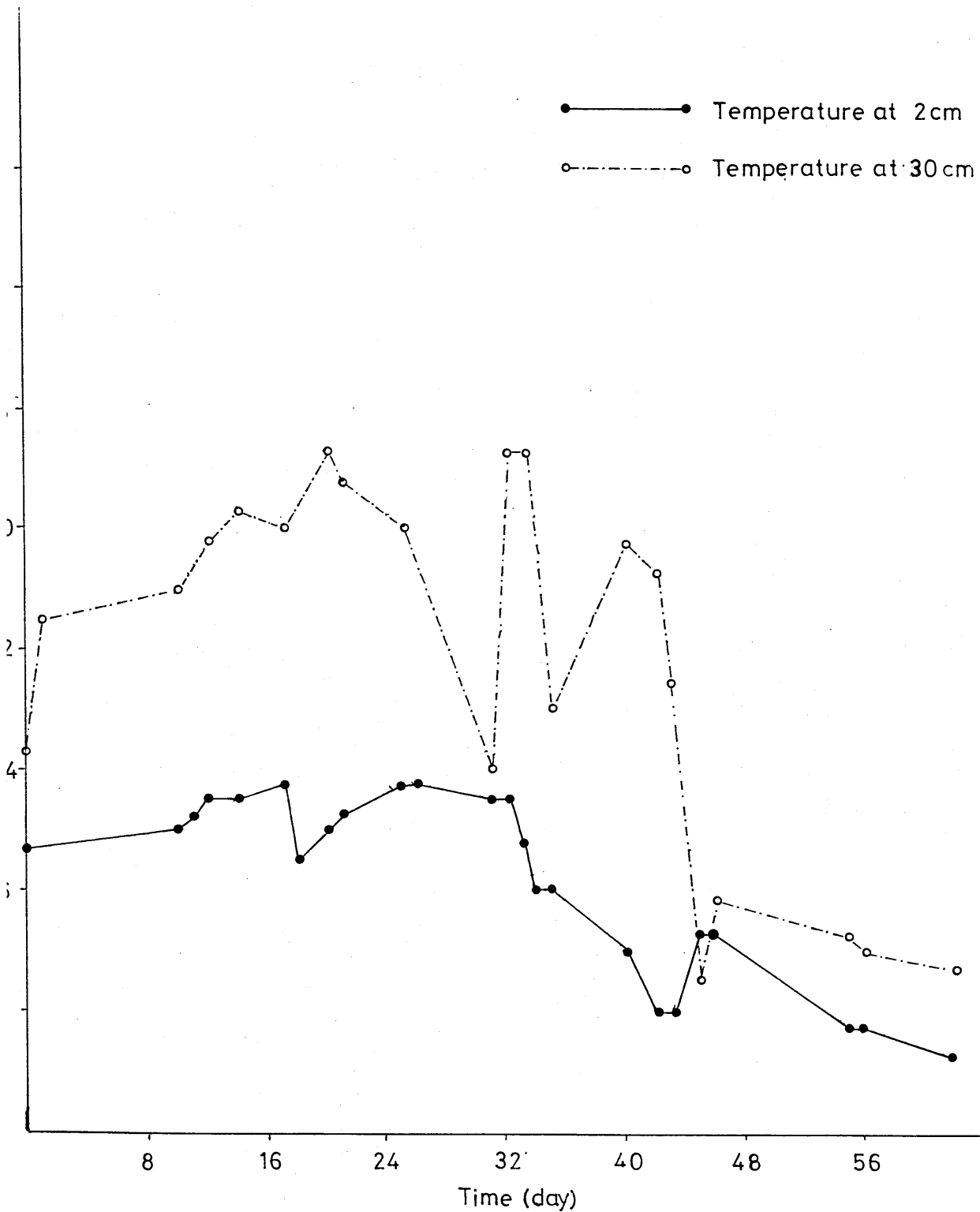


Graph 74.3.3. Growth of Individual Earthworms in Aged and Composted Bore Place Digested Solids

Graph 74.34. Growth of Eight Earthworms in
Aged and Composted Digested Bore Place
Solids



Graph 74.35. Temperature of Composting
Bore Place Digested Solids at Two Depths



7.4.4) Discussion of Results; The Effect of Anaerobic Digestion on Bore Place Solids.

Taking the values from time zero, when the materials were freshly collected, the results presented here can be related to those of NIRD solids from section 7.2. The results show that undigested solids have a higher T.S. value than digested solids. This is due to the breakdown of total solids in slurry on anaerobic digestion, mainly the cellulose fraction (Robbins *et al* 1983). For NIRD solids this process only led to a reduction of T.S. for the liquid fraction. The difference may be caused by the longer than usual 40 day retention time of the Bore Place digester, which gave anaerobic bacteria time to begin the process of breaking down fibrous material of a larger particle size, thus reducing the amount of material which enters the solid fraction on separation. It is interesting to note that Hill (1983) calculates that in terms of V.S. reduction and methane production the theoretical minimum retention time for mesophilically digested dairy cattle solid is 8 days, although in practice the normal time is 15-20 days (Friman 1985).

The biggest difference between the two solids is the NH_3 and NH_4^+ ion concentrations which are three times higher in digested solids. Theoretically there is no loss of nitrogen during anaerobic digestion, only methane, water and some sulphur compounds. Hobson (1985) states that there is very little change in the form of N during digestion, although Hill (1980) noted an increase in $\text{NH}_3\text{-N}$ from 657.5mg/l to 2042.4 mg/l on anaerobic digestion of high solids concentration dairy manure. This apparent anomaly can be explained if very rapid changes occur in the period directly after digester effluent discharge. A large obligate anaerobic micro-flora develops during the process of anaerobic digestion which will be exposed to air on discharge, changing the microhabitat and causing microbial death and decay and leading to the rapid mineralization of microbial N to $\text{NH}_3\text{-N}$.

In contrast digested solids have lower kjeldahl N values. There are several possible mechanisms involved to account for this. Firstly the high pH of digested slurry will

push a proportion of $\text{NH}_4^+\text{-N}$ into the free ammonia phase, and so increase the opportunity for N loss through volatilisation. Secondly the mineralization of N on digestion allows a higher proportion into the liquid fraction, as seen for NIRD slurry on separation. Thirdly it is likely that organic N as fibre bound protein and microbial biomass is more likely to enter the solid fraction of undigested solids because of the microbial degradation processes of anaerobic digestion.

The high ionic conductivity measured in digested solids may be caused by the mineralization of N and P and the apparent increase in other mineral ions through the loss of organic material on anaerobic digestion.

The lower value of total organic carbon recorded in digested solids is in variance with the values presented in section 7.2.4 for NIRD digested and undigested solids, and again may be due to the 40 day retention time of the Bore Place slurry, allowing a greater increase in organic carbon breakdown. The carbohydrates, fats and proteins will be broken down quickly in both digesters (Stafford 1980), but the longer retention time of Bore Place slurry will allow an increase in the breakdown of cellulose and hemi-cellulose, especially that shielded by lignin. There would therefore be a lower percentage of carbon in digested solids. Both undigested and digested solids have similar C/N ratios, the loss of carbon as methane and the N losses described above balancing each other out.

7.4.5) Changes Over Time in Bore Place Digested and Undigested Solids.

The main changes over time in the two treatments refer to loss of carbon and nitrogen. Digested solids maintains higher NH_3 and NH_4^+ values throughout the aging process. The mean pH values for digested and undigested solids over the 45 day period are 8.5 and 8.3, favouring the loss of N as ammonia through volatilisation. For both types of solids the majority of losses take place in the first 12 days. Nitrification of ammonia to NO_2^- and NO_3^- ions is unlikely to be a source of ammonia loss, at least initially. High NH_3

levels, high temperatures and high pH values inhibit the nitrification process (Sharma and Ahlert 1977). Debertoldi *et al* (1982) noted the initiation of nitrification only after 20 days of composting. If any nitrate is formed it may still be lost through denitrification in anaerobic pockets, as occurs in the soil (Fillery 1983).

Undigested solids suffer a greater loss of carbon, perhaps from a fraction already lost from digested solids on digestion. Because there is little change in the total kjeldahl N values for the two treatments, the further loss of C from undigested solids reduces the C/N ratio to give a final value of 16.3 compared with 19.4 for digested solids.

7.4.6) Changes in Digested Solids on Aging and Composting.

Whether the process of decomposition occurs through mesophilic or thermophilic micro-organisms depends on the physical layout of the solids; whether they are piled in a heap or spread out to increase the surface area/volume ratio. The three main differences generated in the piles are temperature, the groups of micro-organisms present and the amount of surface area of the solids exposed to the atmosphere.

Digested solids have a high ionic conductivity and NH_3 and NH_4^+ -N concentrations, and low total organic carbon and kjeldahl N values before the imposition of aging and composting treatments on the solids.

The material has responded to the two treatments in the following ways; the levels of total kjeldahl N and organic carbon have fallen faster in composted solids, as has the V.S. value, whilst the ionic conductivity value has risen higher. These values indicate a higher level of activity of decomposing organisms on composting than aging. The NH_3 and NH_4^+ -N values fall to low levels, but aged solids have a four times higher concentration after 45 days, suggesting the higher temperatures generated by composting have increased the rate of ammonia volatilisation.

At the end of 45 days composted solids have lower carbon and nitrogen values, indicating potentially lower amounts of earthworm nutrients, which coupled with higher conductivity values in composted solids allows a theoretical assessment that aged solids are going to be capable of supporting higher earthworm growth rates.

7.4.7) Comparison Between Earthworm Growth in Bore Place Digested and Undigested Solids.

Students t-test was used to compare the mean earthworm weight in the two materials at different time intervals for both one and eight earthworms. Significance was determined at the 5% level.

For individual earthworms the mean weight was significantly higher for undigested solids on days 10, 24 and 49. Thereafter there was no significant difference in the mean weights of earthworms cultured in digested or undigested solids, although those in digested solids were higher. These results indicate a faster initial rate of growth of individual earthworms in undigested solids.

In contrast, for eight earthworms the mean weight of earthworms in undigested solids is significantly higher for every time period of the experiment. The pattern of earthworm growth response in Bore Place undigested and digested solids is similar to that in section 7.3.4, whereby better earthworm growth is obtained in undigested solids, and the difference is enhanced at higher earthworm stocking densities. The difference between undigested and digested solids in terms of earthworm culture must either be through differing environmental factors or nutritional availability for earthworms. As discussed in section 7.4.4 digested solids have a higher general ionic conductivity value and specifically a higher NH_3 and $\text{NH}_4^+\text{-N}$ concentration, known to be deleterious to earthworms. However, the 40 day digester retention time has also been shown to have reduced the more easily degradable fibre within the slurry passing through it, leaving a higher percentage of

carbon present in the separated digested solids as resistant lignocelluloses and so reducing the microbial and earthworm nutrient content of the material.

The greater significant differences between the two materials at higher stocking densities may therefore be ascribed to either an ameliorating effect of greater numbers of earthworms in the same amount of material, which influences undigested solids to a greater degree, or more likely the greater demand for an organic carbon food source by higher earthworm stocking densities exposes the nutritional deficiencies of digested solids.

The 40 day slurry retention time is atypical, but merely exacerbates a trend of organic carbon breakdown which occurs to a lesser extent at more usual retention times. The less significantly higher earthworm weights in undigested and digested solids may be a product of a lower retention time, and also possibly a stocking density of only four earthworms may not have been sufficient to allow nutrition to be a great enough limiting factor to earthworm growth in digested solids.

7.4.8) The Effect of Aging and Composting on Earthworm Growth in Digested and Undigested Bore Place Solids.

For individual earthworms using Students t-test at the 5% significance level, aged solids have a significantly higher mean weight at day 10 and day 24. However at day 65 and day 84 composted solids produce a higher mean earthworm weight. However for days 95 and 121 there is no significant difference between the mean earthworm weights. For eight earthworms composted solids supports a significantly higher mean earthworm weight for days 10 and 24 but thereafter there is no significant difference between the mean earthworm weights between the two materials.

In section 7.4.5 the low nutritional status of digested solids was discussed. This is because during digestion available carbon has been stripped from fibre by anaerobic cellulolytic

bacteria, and the mineralization and volatilization of N after the digested slurry has been discharged. Such changes over-ride the differences brought about by the composting and aging process reported in section 7.4.6 whereby composting further reduces the nutritional status of the material over aging. There is little differential earthworm response. These results conflict with those obtained in section 4.2 where digested solids supported a greater earthworm biomass. It is likely that the 40 day retention time of the digester allowed a far greater anaerobic degradation of the slurry than occurred earlier, so producing a nutritionally poorer material for earthworm growth.

Chapter Eight

The Effects of Manipulating the Earthworm Environment on Growth Characteristics

8.1) Introduction to the Chapter.

The process of anaerobic digestion could be seen as physico-chemical manipulation of the cattle solids that make up both the environment and nutrition of *E.fetida*. However, as detailed in chapter seven, changes brought about by the activity of anaerobic micro-organisms and mechanical separation are many, so that links between changing physico-chemical factors and earthworm growth responses are difficult to discern. The work in this chapter is concerned with altering specific physico-chemical factors of the earthworm environment so as to be able to better relate the changes to earthworm growth in a co-ordinated manner. This chapter includes work on altering the C/N ratio of separated solids, using separated liquids as a nutrient source for differing bulking agents, investigations into simple artificial media for earthworm growth which could be used to study earthworm/physico-chemical interactions.

8.2) The Effect of Cellulose Additions to Separated Solids on the Growth of *E.fetida*.

8.2.1) Introduction.

Cellulose and hemi-cellulose are the main polysaccharides in the fibre of cattle waste (Martin *et al* 1983) derived from both the bedding and undegraded foodstuffs in the diet. Cellulose represents the main carbon source in cattle solids for micro-organisms and therefore earthworms, and so an understanding of the role of cellulose in the nutrition of earthworms is an important part of the relationship between earthworms and their organic waste environment. One way to gain an insight into this is through the addition of cellulose in varying amounts to cattle solids and the subsequent effects it has on earthworm growth rates. The addition is carried out at four rates (5%, 10%, 20% and 30%) for separated digested solids from Bore Place and NIRD, with controls of digested and undigested solids. Digested solids have been chosen because of the breakdown of fibre-C during anaerobic digestion may lead to differences in the C/N ratio.

Carbon and nitrogen are the two most important elements in the nutrition of all but a few living organisms and the ratio of the two gives a useful indication to the nutritional value of a material, and the demand for the two elements occur in a certain ratio. Waksman and Heukelekian (1925) found cellulose decomposing fungi consume nitrogen in a definite ratio to the amount of cellulose, one unit of N for every 30-33 units of cellulose.

The effect of the C/N ratio in composting has been reviewed by Gotaas (1956) for municipal wastes. Since decomposition is carried out by living organisms whose nutritional requirements are met by 25-35 units of carbon for every unit of nitrogen, it follows that the optimum C/N ratio for composting is 25-35, allowing all carbon and nitrogen to be efficiently utilised. It is important to note that for it to be meaningful the C/N

ratio must refer to carbon and nitrogen that is nutritionally available for micro-organisms, and thus earthworms. The carbon in lignin, for example, which becomes only limitedly available for micro-organisms after a long time period (Grushnikov and Antropova 1975). Nitrogen which has become chemically incorporated into humus (Haider *et al* 1965), is essentially unavailable and has no immediate role in the decomposition process, although would be taken into account in a general C/N ratio value.

If the C/N ratio is lower than the optimum there is an excess of nitrogen, and carbon is the limiting factor to decomposition. In this case micro-organisms have all the N necessary for the utilisation of carbon as an energy source, and so decomposition occurs at a fast rate with a high CO₂ evolution rates. However, there is an increased loss of N which is not incorporated into the microbial biomass. This occurs through the volatilisation of ammonia and the denitrification of nitrate in anaerobic microsites which may develop because of the high microbial respiration rates. There may also be leaching losses of nitrate in unprotected compost heaps, as nitrification is known to occur more rapidly in materials with a low C/N ratio (Jenkinson 1928).

When the C/N ratio is higher than the optimum, nitrogen is limiting, and not all the carbon present can be used as an energy source. All available will be incorporated into the microbial biomass, and will only be released when the micro-organisms die. Thus the microbes must go through several life-cycles releasing N for subsequent microbial incorporation before all the available carbon is decomposed. A high C/N ratio therefore causes decomposition to occur more slowly, with all available nitrogen 'locked up' in the microbial biomass.

There are other specific effects that may occur on changing the C/N ratio, but these are dealt with in detail in section 8.2.4 with reference to the results obtained.

8.2.2) Materials and Methods.

Digested and undigested solids were obtained from Bore Place and NIRD and aged in the laboratory for two weeks until acceptable to earthworms. The materials were analysed as in section 5.1.3. The moisture content of the solids was adjusted to 85% by the addition of distilled water. 40g wet weight of each solid was placed in 10cm diameter plastic containers, and powdered cellulose (Sigma Chemical company, USA) added at the following rates; 5% wet weight/wet weight, 10%, 20% and 30%. The controls received no cellulose addition and included both the digested and undigested form of the solid from the particular site. For each treatment four *E.fetida* weighing between 0.1 and 0.2g were inoculated with 10 replicates per treatment. The containers were placed in an incubator at $20 \pm 1^\circ\text{C}$ and the earthworm weights sampled every 7-14 days as described in section 4.2.2.

8.2.3) Results.

Table 8.2.3.1 shows the physico-chemical analysis of the two solids and the change in C/N ratio on cellulose addition. Graphs 8.2.3.1 and 2 show growth curves for *E.fetida* in amended Bore Place and NIRD cattle solids. Graph 8.2.3.3 shows the peak weights from the growth curve. Graph 8.2.3.4 shows the loss of weight of earthworms cultured in pure moistened cellulose. Table 8.2.4.1 shows a t-test matrix comparing the mean earthworm growth rates in the various treatments.

Table 8.2.3.1.

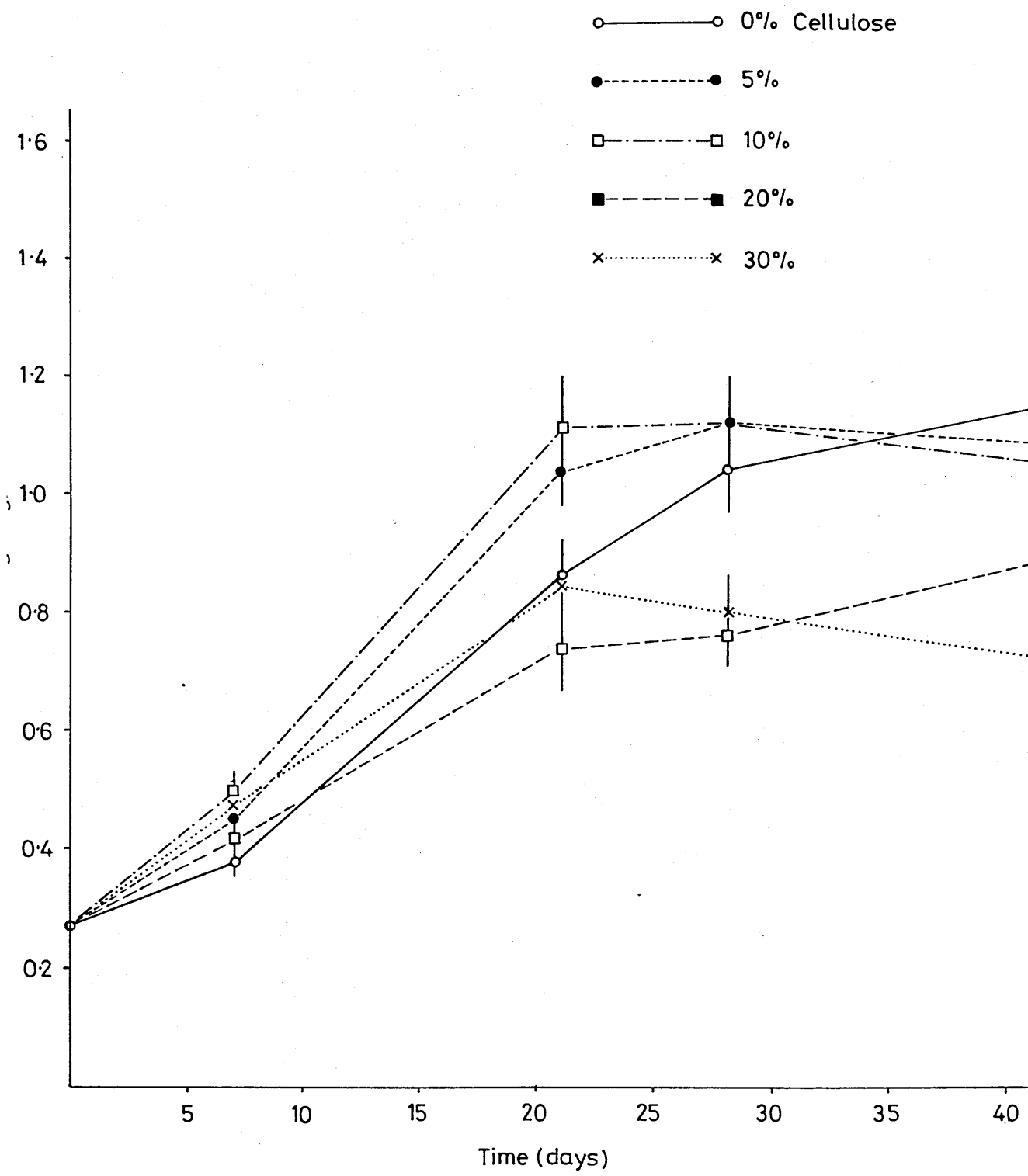
Physico-Chemical Analysis of Digested Solids and Cellulose.

Variable	Digested NIRD Solids	Digested Bore Place Solids
pH	8.5	8.9
Moisture Content (%)	81.3	77.4
Ionic Conductivity ($\mu\text{S}/\text{cm}$)	4530	5200
$\text{NH}_3\text{-N}$ (mg/g dry wt)	<0.01	<0.01
NH_3 and $\text{NH}_4^+\text{-N}$ (mg/g dry wt)	0.09	0.05
Total Kjeldahl Nitrogen (%)	2.3	1.8
Total Organic Carbon (%) (Walkley-Black Corrected)	43.9	41.2
C/N Ratio	19.1	22.9

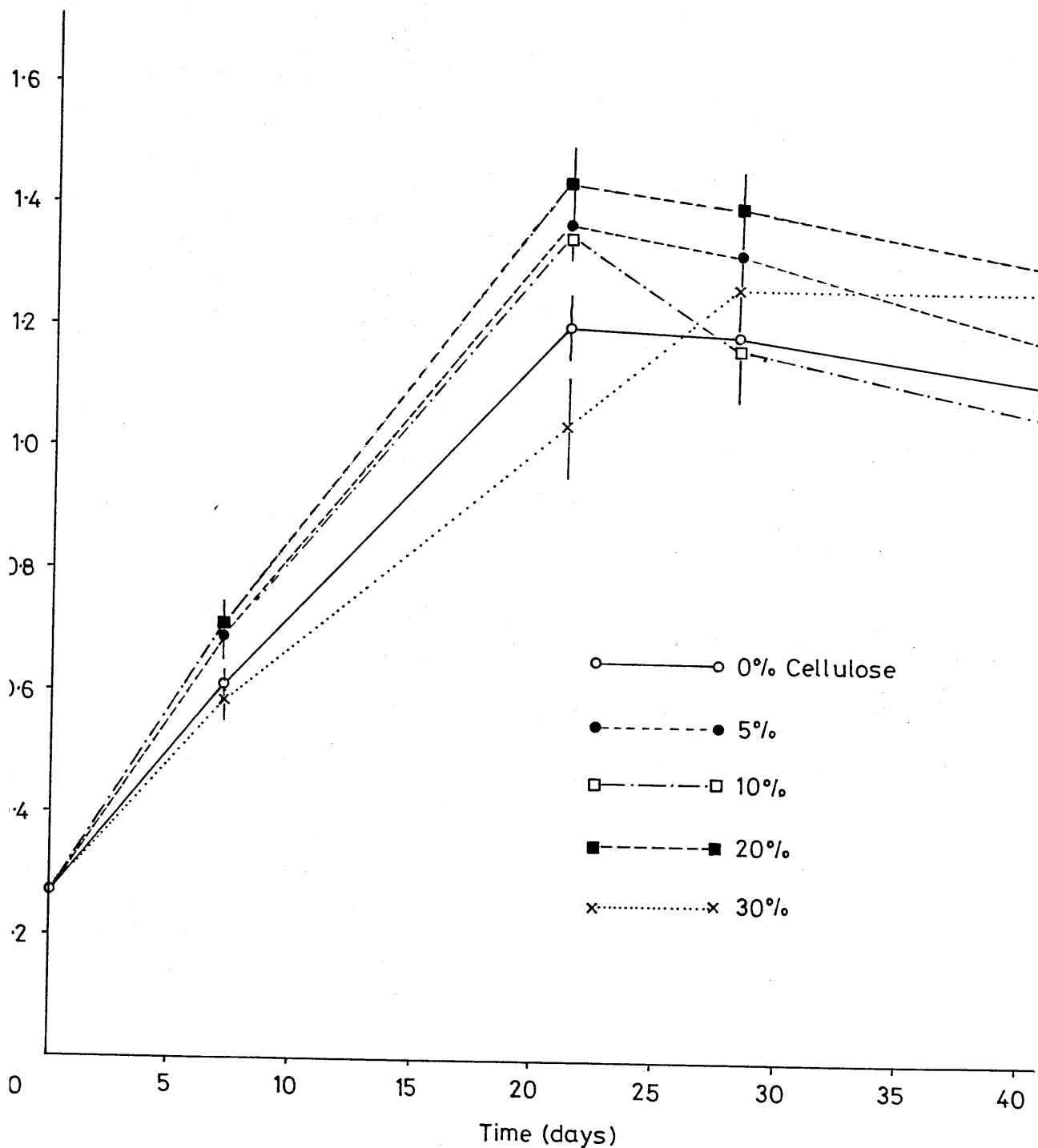
Change in C:N Ratio on Cellulose Addition.

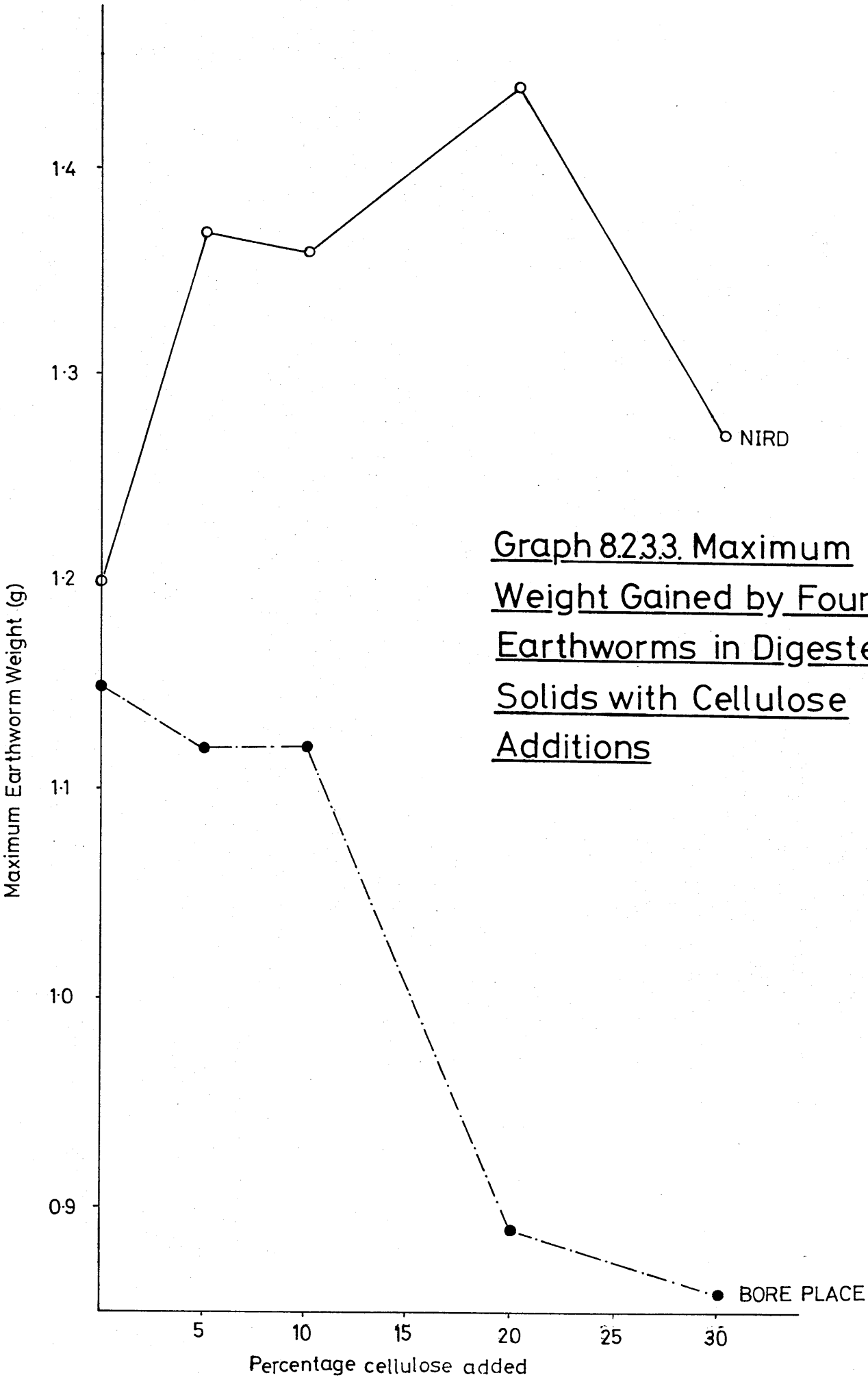
Rate of Cellulose Addition	0%	5%	10%	20%	30%
C/N Ratio of NIRD Solids	19.1	19.5	19.9	20.6	21.4
C/N Ratio of Bore Place Solids	22.9	23.3	23.8	24.7	25.6

Graph 82.3.1. Growth of Four Earthworms in Digested Bore Place Solids with Additions of Cellulose to alter the C/N Ratio



Graph 8.23.2. Growth of Four Earthworms in Digested NIRD Solids with Additions of Cellulose to alter the C/N Ratio





Graph 8.23.3. Maximum Weight Gained by Four Earthworms in Digested Solids with Cellulose Additions

Graph 8.23.4. The Growth of Individual
E. foetida in Pure Cellulose Powder at 80%
Moisture Content

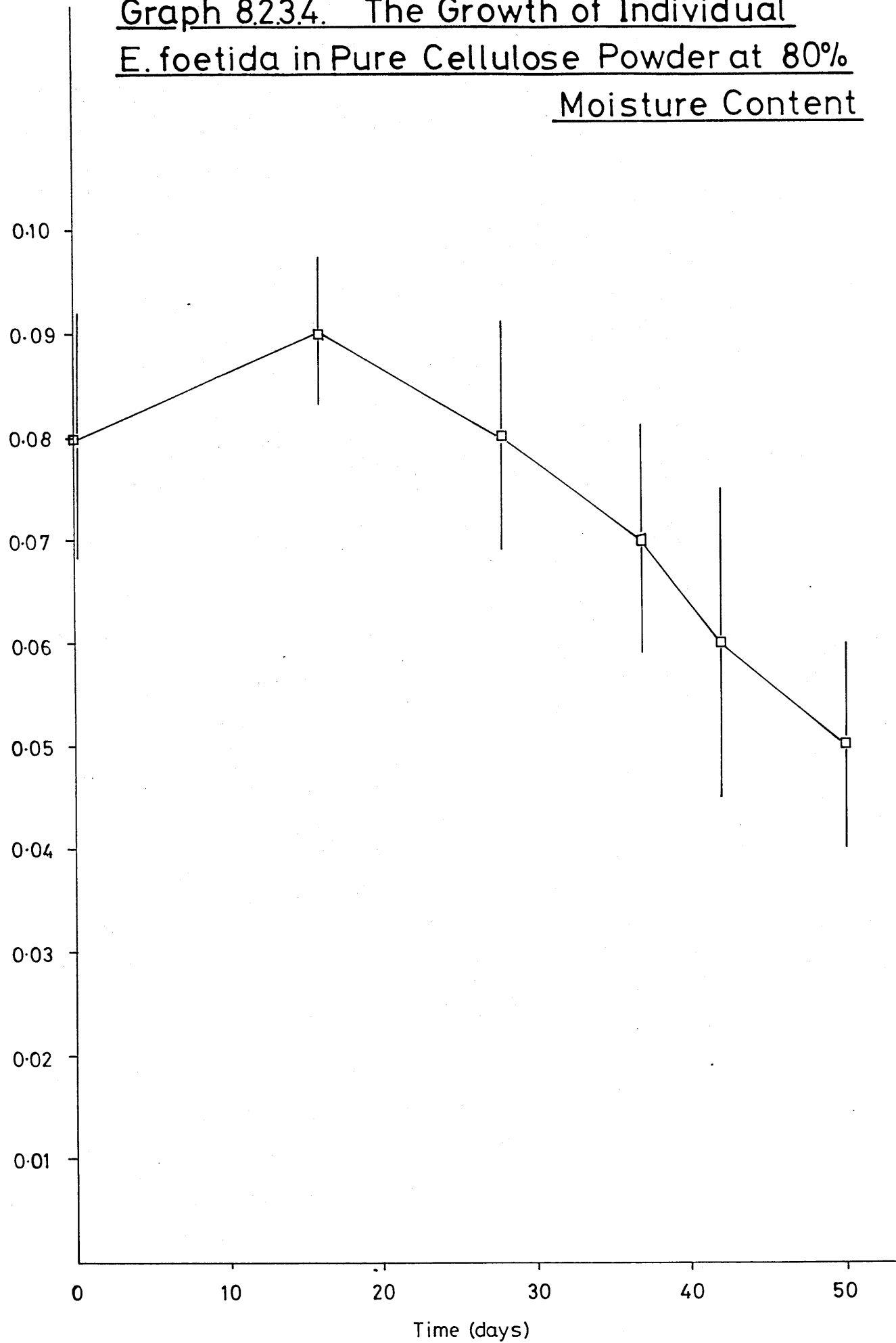


Table 8.2.4.1. T-Test matrices for the Mean Weight of Earthworms on the Addition of Cellulose to NIRD and Bore Place Digested Solids at Four Rates.

X = An irrelevant comparison, blank square = no significant difference.

Arrow points to significantly different value (p=0.05).

Day 7

BPC	↓								
N 5%	←	X							
N 10%		X							
N 20%		X							
N 30%		X	↓						
BP 5%	X	←	↓	X	X	X			
BP 10%	X	←	X	↓	X	X			
BP 20%	X		X	X	↓	X			
BP 30%	X	←	X	X	X				
	NC	BPC	N 5%	N 10%	N 20%	N 30%	BP 5%	BP 10%	BP 20%

Day 21

BPC	↓								
N 5%	←	X							
N 10%	←	X							
N 20%	←	X							
N 30%		X	↓	↓	↓				
BP 5%	X		↓	X	X	X			
BP 10%	X	←	X	↓	X	X			
BP 20%	X		X	X	↓	X	↓		
BP 30%	X		X	X	X	↓	↓		
	NC	BPC	N 5%	N 10%	N 20%	N 30%	BP 5%	BP 10%	BP 20%

Table 8.2.4.1. T-Test matrices for the Mean Weight of Earthworms on the Addition of Cellulose to NIRD and Bore Place Digested Solids at Four Rates (continued).

X = An irrelevant comparison, blank square = no significant difference.

Arrow points to significantly different value (p=0.05).

Day 28

BPC									
N 5%		X							
N 10%		X	↓						
N 20%	←	X		←					
N 30%		X							
BP 5%	X		↓	X	X	X			
BP 10%	X		X		X	X			
BP 20%	X	↓	X	X	↓	X		↓	
BP 30%	X		X	X	X	↓		↓	
	NC	BPC	N 5%	N 10%	N 20%	N 30%	BP 5%	BP 10%	BP 20%

Day 42

BPC									
N 5%		X							
N 10%		X	↓						
N 20%	←	X	←	←					
N 30%	←	X		←					
BP 5%	X			X	X	X			
BP 10%	X		X		X	X			
BP 20%	X	↓	X	X	↓	X	↓		
BP 30%	X	↓	X	X	X	↓	↓	↓	
	NC	BPC	N 5%	N 10%	N 20%	N 30%	BP 5%	BP 10%	BP 20%

8.2.4) Discussion of Results.

Cellulose is a carbon source that earthworms are able to utilise under certain conditions (Flack and Hartenstein 1984). Earthworms can survive in pure cultures of cellulose (see graph 8.2.3.4), but slowly lose weight, lacking a N source for biomass gain (Morgan 1986). Hartenstein (1984) found a significantly reduced percentage weight loss for *E.fetida* cultured in cellulose compared to ashed loam. This implies that earthworms gain some nutritional benefit from cellulose, and relates to studies that suggest that the gut of *E.fetida* has an independent cellulase enzyme (Tracey 1951, Parle 1963b).

From these experiments, a gain in maximum earthworm biomass from cellulose addition will only be seen if metabolisable carbon is a limiting nutritional factor. Assuming that environmental factors such as pH, ionic conductivity and ammonia concentration are not inhibiting earthworm growth then carbon can only be utilised if there is a sufficient source of N. Therefore the absolute values of C and N and the C/N ratio of the solid can be used to determine its nutritional quality. As has already been discussed (section 3.1.5) a proportion of cellulose in slurry and thus the separated solids is broken down on digestion. However, the mineralization of N which has been shown to occur in digested slurry on discharge from a digester, and the subsequent ammonia volatilisation, tends also to reduce the total levels of nitrogen in the separated digested solids, so that the differences in the C/N ratio between digested and undigested solids are not as great as one would expect.

No pattern of earthworm growth response to cellulose addition emerges (graphs 8.2.3.1 to 3). A matrix of Students t-tests were carried out to statistically compare the mean earthworm weights on cellulose additions at varying rates and the control within both Bore Place and NIRD solids, and comparing relevant cellulose additions between Bore Place and NIRD solids. The results are shown in table 8.2.4.1

To summarise these results, earthworms cultured in NIRD solids at the varying cellulose additions have statistically higher mean earthworm weights than Bore Place solids, but the number which are statistically greater is reduced over the time period of the experiment. This reflects the greater earthworm carrying capacity of NIRD solids over Bore Place material, as noted in chapter five.

The addition of cellulose to NIRD solids does not produce consistent results, but at each time interval at least one rate produces a significantly higher mean earthworm weight than the control, and on day 21 5, 10 and 20% cellulose additions produce a significant increase in earthworm weight. For Bore Place solids a similarly inconsistent picture emerges. On day 7, 5%, 10% and 30% additions increase the mean earthworm weight over the control. However these increases are not maintained and on day 28 the 20% addition rate causes an inhibition of earthworm growth over the control, and on day 42 both 20% and 30% additions have a significant inhibitory effect.

In graph 8.2.3.3 the maximum biomass achieved by earthworms at each rate of cellulose addition has been plotted for the solids at the two sites in order to clarify the earthworm response. Two different patterns emerge; in Bore Place solids increasing additions of cellulose cause successive decreases in the maximum earthworm weight obtained, whereas additions of cellulose to NIRD solids increase the maximum weight gain over the control, peaking at 20% cellulose additions. NIRD solids have a higher percentage kjeldahl nitrogen value than Bore Place solids (2.3% compared with 1.8%) and so a lower C/N ratio (19.1% compared with 22.9%). The growth results indicate that the C/N ratio of 19.1% is lower than the optimum and so extra cellulose additions can be effectively utilised. This is supported by the fact that the optimum cellulose addition in NIRD solids produces a C/N ratio of 22.9, the same value as that of the Bore Place control, and suggests that earthworms can optimally utilise carbon and nitrogen for growth at a ratio of approximately 23. This value is in agreement with Neuhauser *et al* (1984) who cultured one or two earthworms in activated sludge with added cellulose to provide a range of C/N ratios

from 9 to 125. The highest percentage weight gain over a two week period was at a ratio of 23.

Gotaas considers 25-35 as the optimum C/N ratio for composting, based on the knowledge that aerobic micro-organisms require one unit of N for every 25-35 units of C for optimum growth. The results presented here suggest that a C/N ratio above 23 reduces earthworm growth, a value slightly below that of Gotaas. As discussed in section 3.3.1 the optimum C/N ratio refers to available carbon and nitrogen. The cattle bedding at both sites is wood based, with a high proportion of lignin. In short term experiments such as this such carbon will be unavailable to earthworms and micro-organisms, although it will be included in the corrected Walkley-Black value of total carbon. The available C/N ratio is therefore lower than the values recorded, making the disparity between the recorded optimum in these experiments and the theoretical optimum greater.

One may therefore speculate that there are some inhibitory effects related to the addition of cellulose to the separated solids. These can include an increase in the rate of microbial respiration, and thus an increased possibility of anaerobic sites developing within the solids. There is the possibility that organic acids may be formed as the breakdown product of respiration, with consequent changes in the pH. This occurs in the initial stages of the composting process, when easily degraded materials including cellulose are broken down causing a fall in the pH (Gray and Biddlestone 1971). The addition of a single nutrient source in relatively high concentrations may stimulate high levels of single microbial species which may release toxins or toxic metabolic by-products into the separated solids which may inhibit earthworm feeding or increase environmental stress. This is discussed further in section 8.4.

8.3) The use of Separated Liquids to Support Earthworm Growth.

8.3.1) Introduction.

The mechanical separation of animal slurries produces a stackable, compostable solid which is well suited to earthworm culture. However, approximately 60% of the slurry enters the liquid fraction, depending on the dry matter of the input slurry (Pain *et al* 1978), and contains a major proportion of the nutrients and microbial biomass. On farms operating mechanical separators as part of their waste management system, the liquid fraction is normally stored in lagoons prior to landspreading, where its low solids content, pumpability and nutrient content can be taken advantage of.

However, it is possible to utilise this fraction of the slurry for earthworm culture, by combining it with a carbonaceous bulking agent to produce a material of the correct physical consistency. The separated solids increase the moisture content and increase the C/N ratio of carbonaceous materials and therefore make them amenable to microbial breakdown and so allow them to support an earthworm population. The bulking agent in turn increases the availability of oxygen to the separated liquid for aerobic microbial breakdown.

This kind of system could be beneficial if from a separated liquid and a material such as straw a horticultural growing medium and earthworm biomass could be produced with a greater value than that of separated liquids as a landspread fertilizer, and the value of straw which is likely to be marginal or even negative. All agricultural wastes can be defined as materials in the wrong place at the wrong time, and it is possible that farmer could have a surfeit of separated liquid and a carbonaceous material that he could upgrade.

As well as potential economic benefits for a farmer, the study of the addition of separated

liquids to inert bulking agents can also prove useful understanding the nutritional and the physico-chemical requirements of *E.fetida* from organic wastes, as the separated liquid and the bulking agent represent two fractions of the material required for successful earthworm growth; a source of carbon, nutrients and micro-organisms.

Four bulking agents are used, peat, wheat straw, newspaper and woodchips, which all have different types and proportions of carbon bearing compounds, and different physical structure. Peat has a high proportion of carbon in highly polymerised 'humus' molecules, with some associated mineral ions (Weiringa 1963). Woodchips, the bedding used at Bore Place, contains hemi-cellulose, cellulose and lignin in a matrix of all three molecules to form the original structure of the wood. Straw is similar, but has a lower proportion of lignin, the main source of tensile strength in wood. Newspaper is also a mix of cellulose, hemi-cellulose and lignin, but with the ligno-cellulose matrix broken down to a great extent by the papermaking process and containing added impurities from news ink and other chemicals.

8.3.2) Materials and Methods.

Six bulking agents were initially investigated, perlite, vermiculite, straw, woodchips, newspaper and peat. Initial earthworm mortality experiments were conducted to discover if any pretreatment of the liquid was required, and to find the optimum moisture content of the liquid/solid admix. Raw liquids, even if allowed to age for two weeks were toxic to adult *E.fetida*. However, when aerated for 28 days and then allowed to age for one week with the bulking agent the material was acceptable. Table 8.3.3.1 shows the analysis of separated liquid before and after aeration. These suggest that the high biological oxygen demand (BOD) was a likely cause of earthworm mortalities in the raw liquid, although the ionic conductivity and ammonium ion concentration were also high.

When solid/liquid mixes were made up at two moisture contents, 85% caused some

mortalities in all mixes, but 80% was acceptable to earthworms.

No earthworms survived in mixes with perlite or vermiculite, and so they were dropped from the final experiment. This consisted of individual *E.fetida* cultured in peat, straw, woodchips or newspaper with the addition of separated liquid, aerated for 28 days. The mixes were adjusted to have 60g wet weight per container at 80% moisture content, and allowed to age for one week. For each treatment 10 replicates were inoculated with individual hatchlings of *E.fetida*. The experimental procedure continued as in section 3.2.

In addition a secondary experiment was set up as above except that whole and macerated woodchips were used, ground in a 'Braun' grinder for 45 seconds to reduce the particle size of the woodchips. The experiment was to determine the effect of increasing the surface area of the woodchips on earthworm growth.

8.3.3) Results

Table 8.3.3.1 shows the change in physico-chemical variables on aeration of separated liquid. Graph 8.3.3.1 shows the growth of individual *E.fetida* in four bulking agent/separated solid mixes, and graph 8.3.3.2 shows the growth of individual *E.fetida* in whole and macerated woodchips. Table 8.3.3.2 shows the results of Students t-test on mean earthworm weights cultured in the bulking agent mixes. Photograph 8.3.3.1 shows microbial growth in a pot containing straw and separated liquid.

Table 8.3.3.1.

Changes in some physico-chemical characteristics of
separated solids on aeration.

Volume of liquid aerated = 10 litres

Rate of air flow = one litre per minute

Maintained at ambient laboratory temperature

Variable	Initial	After 28 days
Moisture content (%)	4.4	4.7*
Volatile solids (%)	65.5	48.7
Ionic conductivity ($\mu\text{S}/\text{cm}$)	29200	5400
NH_3 and $\text{NH}_4^+\text{-N}$ (mg/l fresh weight)	493	37
pH	7.8	8.3
B.O.D. (mg/l)	25500	7750

* Moisture content adjusted over course of aeration experiment
to make up for evaporative losses

B.O.D. measured as in HMSO (1972)

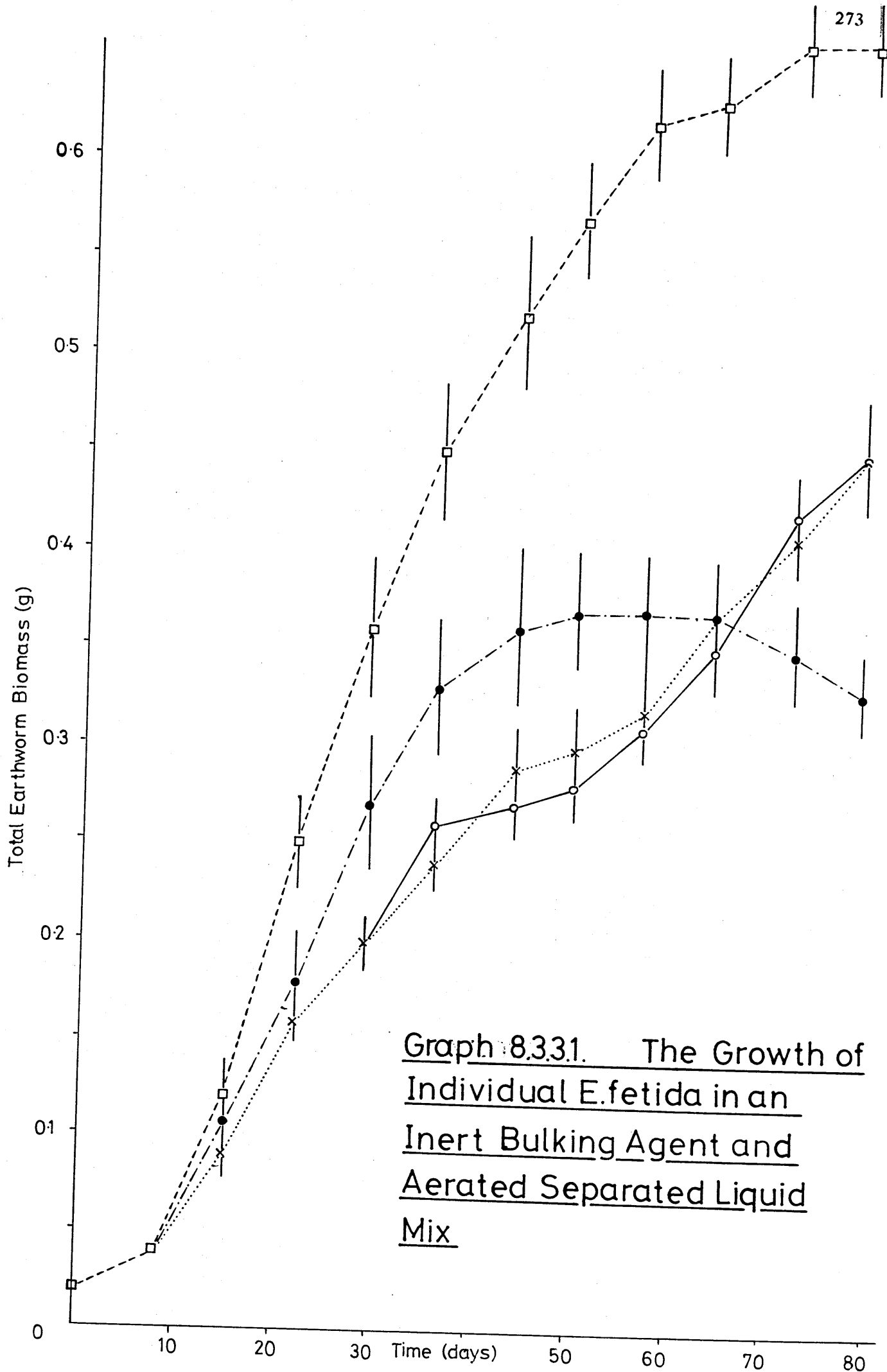
Table 8.3.3.2.

Statistical Analysis of Mean Earthworm Weight in Four
Bulking Agents and Separated Liquid, Using Students t-test.

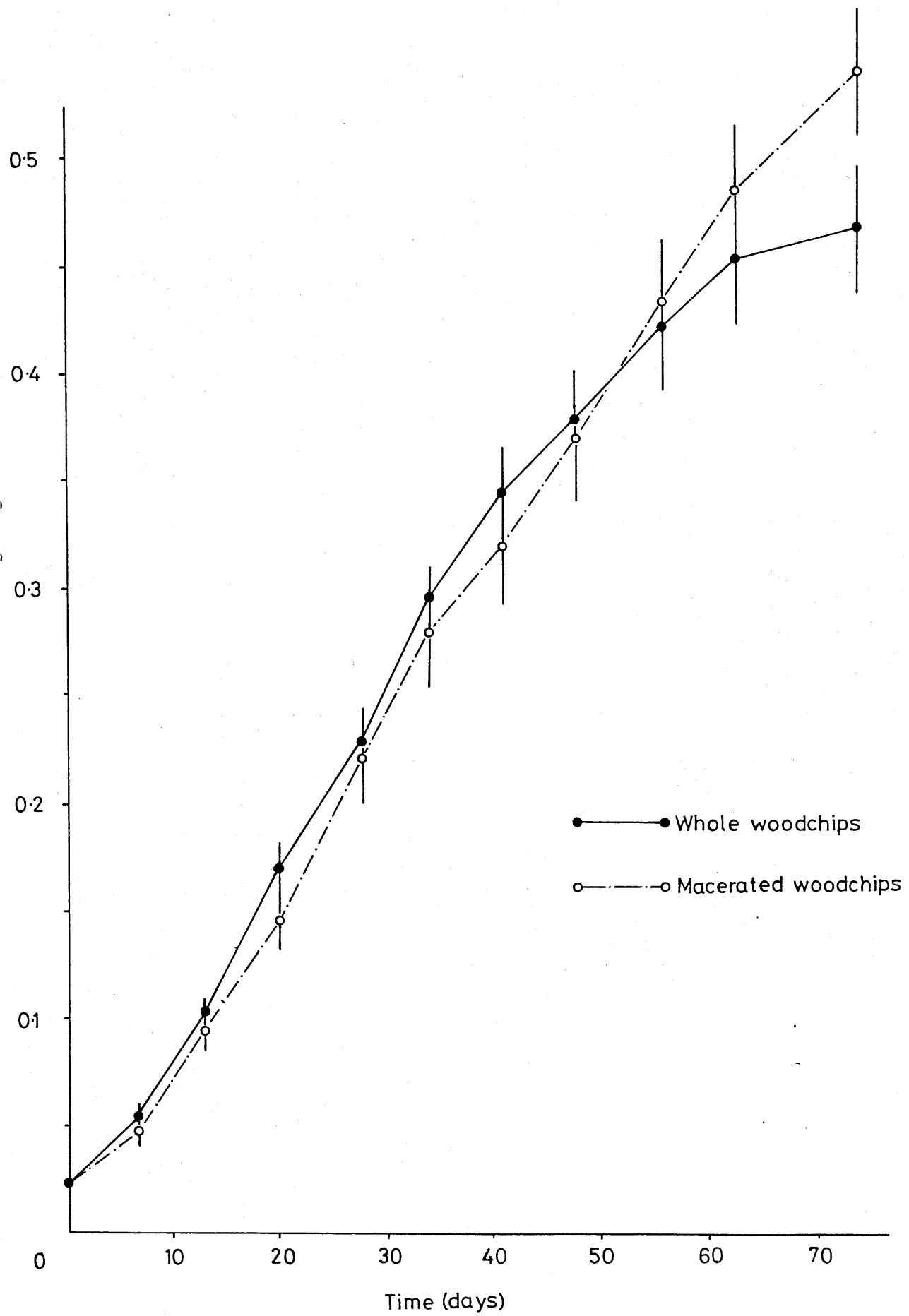
New = Newspaper
Pea = Peat
Str = Chopped Straw
Woo = Woodchips

Str/Woo = Mean earthworm weight in straw is significantly higher
than mean earthworm weight in woodchips at the 5%
level of significance.

Day 0	-	-	-	-	-
Day 8	-	-	-	-	-
Day 15	-	-	-	-	-
Day 22	-	-	-	Str/New	Str/Woo
Day 29	-	-	Pea/New	Str/New	Str/Woo
Day 36	-	-	Pea/New	Str/New	Str/Woo
Day 44	Str/Pea	Pea/Woo	Pea/New	Str/New	Str/Woo
Day 50	Str/Pea	Pea/Woo	Pea/New	Str/New	Str/Woo
Day 57	Str/Pea	Pea/Woo	-	Str/New	Str/Woo
Day 64	Str/Pea	Woo/Pea	-	Str/New	Str/Woo
Day 72	Str/Pea	Woo/Pea	-	Str/New	Str/Woo
Day 79	Str/Pea	Woo/Pea	New/Pea	Str/New	Str/Woo
Day 90	Str/Pea	Woo/Pea	New/Pea	Str/New	Str/Woo

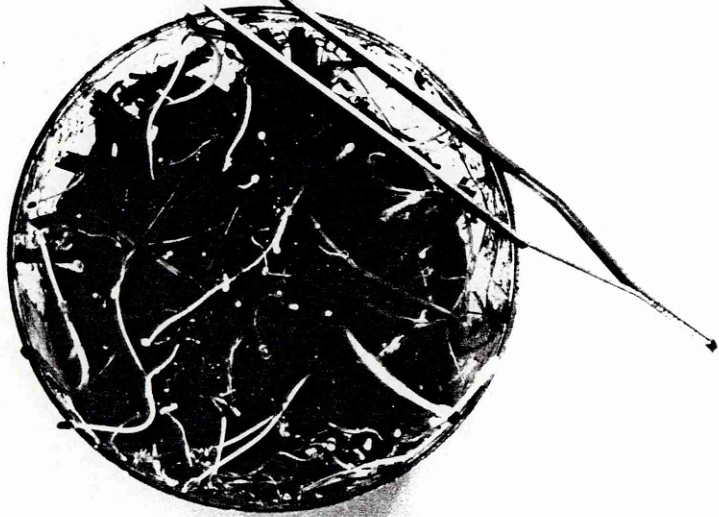


Graph 8.3.3.2. Growth of Individual Earthworms
in Separated Liquid with Whole and Macerated
Woodchips as a Bulking Agent



Photograph 8.3.3.1.

Fungal Growth in Containers of Chopped Straw
Used as a Bulking Agent for Separated Liquid.



8.3.4) Discussion of Results.

The initial trials showed that vermiculite or perlite mixes could not support earthworm growth. These bulking agents differ from others in that they are mineral in origin, and could not therefore contribute to the carbon requirements of the earthworm. However, a nutritionally deprived environment is very unlikely to have caused earthworm mortalities in so short a time period. This would suggest some physical interaction between the absorbant solid and liquid which is deleterious to earthworms.

The initial trials also showed that the admixes caused mortalities at moisture contents of 85%, but not 80%, although the former found to be acceptable to earthworms in the separated solids trials of chapter four. The particle size of separated solids provides a wide range from coarse to fine particles. This allows a range of pore sizes to develop within the solids with the ability to hold large amounts of liquid without creating anaerobic sites within the material. For straw, woodchips and to a lesser extent peat and newspaper, the larger particle sizes allow only large pores to develop which can only hold a certain amount of liquid before becoming anaerobic. This is the most likely explanation of the need to reduce the moisture content of the solid/liquid mix for earthworm survival.

From graph 8.3.3.1 clear differences between the bulking agent/liquid mixes emerge with the straw mix providing the greatest earthworm biomass increase per pot per day over the other bulking agents. This conclusion is supported statistically by Students t-test as in table 8.3.3.2. Straw provides a significantly better medium than newspaper, woodchips and peat for most of the experimental time period.. Peat provides better growth conditions than newspaper and woodchips initially, but by the end of the experiment this situation is reversed.

Quoted values for the hemicellulose, cellulose and lignin fractions for straw, woodchips

and newspaper are given below;

Constituent	Wheat Straw [*]	Newspaper ^{**}	Woodchips ^{***}
Hemicellulose	29.0	8.0	11.0
Cellulose	50.1	84.0	28.0
Lignin	13.7	6.0	28.0

^{*} Anderson and Anderson (1980)

^{**} Bond and Straub (1973)

^{***} for white spruce

From these results one would expect newspaper to produce the best results (as it has the highest proportion of earthworm available carbon and has undergone treatment to break down the ligno-cellulose matrix) followed by straw and then woodchips. However this was not the case, possibly indicating that contaminants of the newspaper, principally the news ink which may contain polychlorinated biphenyls and therefore have have an inhibitory effect on earthworm growth.

Photograph 8.3.3.1 shows strong fungal growth in the wheatstraw/liquid mix, indicating that conditions were correct for the microbial breakdown of polymerised carbon of straw, and so providing a good source of nutrition for earthworm growth. These results can be seen in the light of field scale trials in section 2.6, where poor earthworm growth was observed in straw based farmyard manure with little associated faeces. These results show that with the correct C/N ratio straw can be degraded very quickly and be used as a carbon source for earthworm culture.

With a higher lignin content one would expect the lower growth rates that were observed in woodchips. These results indicate that the woodchips are not totally inert, but can provide a limited source of carbon for microbial and earthworm nutrition. This has implications for the results of earthworm growth in Bore Place separated solids where

woodchips form the majority of fibre of solids after separation.

Earthworms have been shown to be unable to break down the aromatic molecules that make up the structure of peat (Hartenstein 1982) and so in terms of this experiment peat can be seen as a nutritionally inert material. Satchell and Dottie (1984) studied the longevity of *E.fetida* stored in peat, and found that weight loss was lower in peats of low pH. The authors suggest that the low pH inhibited earthworm activity and so they burrowed less, produced less faeces and respired less than in neutral peat where energy output exceeding input, resulting in greater weight losses. In this experiment the pH of the peat was 6.3. The alkaline nature of the separated liquids would tend to neutralise this further, and so it is unlikely that there was any pH mediated inhibition of earthworm in the peat/separated liquid mix of this trial.

Graph 8.3.3.2 shows the effect of macerating woodchips to decrease the particle size on earthworm growth. Using Students t-test there is no significant difference in mean earthworm weight between the two treatments at the final time period, after 74 days, when macerated solids produced the highest mean value. The maceration process has therefore had a limited effect on stimulating earthworm growth. Milling, grinding and cutting are common processes in the chemical industry for dealing with cellulosic materials (Dunlap and Chiang 1980), and Dehority and Johnson (1961) found an increase in the digestibility of cellulose for ruminants on a decrease in particle size. However, to be effective the grinding process must affect the structure of the cellulose and associated lignin to decrease the degree of polymerisation. The grinder used in this experiment did not produce a reduction in particle size of this order.

In contrast Neuhauser *et al* (1980) found a significant increase in percentage weight change in *E.fetida* in manures of different particle sizes mixed with soil. The authors conclude that this is due to an increase in surface area for microbial growth to provide food for *E.fetida*.

8.4) The Creation of an Artificial Earthworm Growth Medium.

8.4.1) Introduction.

In chapter six the importance of physico-chemical factors within separated solids in determining earthworm growth was seen. However the difficulty in relating the influences in earthworm growth to any one or combination of factors was also shown.

In sections 8.2 and 8.3 in this chapter it was found difficult to alter just one set of physico-chemical characteristics within separated solids without changing others. This is because aerobically decomposing cattle solids are formed through a complex interaction between organic and inorganic substrates and a range of different micro-organisms. When earthworms are added to this system they too become involved in a range of interactions which are difficult to study in isolation. This provides the motivation to create a synthetic medium which is chemically defined and in which earthworms will grow, but where the interactions within the medium are much simplified, allowing for experimental manipulation of some of the factors that effect earthworms.

In producing a synthetic medium the minimum requirements to support earthworm growth must be known. This is an area where the literature is not comprehensive. The review of the literature in section 1.4.4 on the nutrition of *E.fetida* shows the importance of micro-organisms in the diet of earthworms, but it is not possible to define which microbial species make up the diet of *E.fetida* from the research carried out so far in this field. In terms of a synthetic medium the requirement is for a mixed microbial culture containing a range of species that *E.fetida* will feed upon. Morgan (1985) found greater earthworm growth in mixed cultures compared to pure cultures of individual microbes. The emphasis in this section is not upon earthworm-microbial interactions but rather earthworm-environmental interactions. The minimum requirement for a synthetic medium

to support a mixed microbial population is a carbon based energy source, a nitrogen source, necessary mineral ions and possibly essential vitamin complexes.

In cattle solids the major long term carbon sources for microbial growth are hemi-cellulose and cellulose. Oils, starches and sugars are unlikely to have survived passage through the ruminant gut, and volatile fatty acids, mainly acetic acid will be rapidly broken down in the aerobic conditions of separated cattle solids. This of course simplifies a very intricate microbial ecosystem. Cellulose provides the simplest basic carbon source on which to base an artificial medium. Hayes (1986) found *E.fetida* flourishing in the mainly cellulose based waste discharge from a paper making factory. If it is used, powdered moistened cellulose also has the advantage of providing a physical substrate which earthworms can freely move in and ingest easily, and can also be utilised as a carbon source (see section 8.2).

Using cellulose as the sole carbon source has the disadvantage of producing a microbial flora consisting mainly of cellulolytic bacteria and fungi. However in this respect the experimental work is different to the earthworm microbial studies of Morgan (1986), Flack and Hartenstein (1984) and Neuhauser *et al* (1980) where cellulose was used as a medium in which to add test microbial species cultured separately. In an artificial medium the micro-organisms present would be specifically using the medium as a nutritional source.

In order to support a flourishing micro-flora inorganic nutrients are required, added in solution, which brings the moisture content of the medium to the required 80-85%. The ions supplied are the minimum needed to satisfy the general requirements of a microbial population. There is a conflict here between the need to allow microbial growth and complicating the make up of the medium. Earthworms have been shown to be sensitive to the concentration of ions in the environment and so the use of the most simple nutrient solution is important to allow further manipulation of both the general ionic conductivity, and also specific ion concentrations in order to judge their effect on earthworm survival,

growth and reproduction, if an artificial medium can be produced which supports earthworm growth in the first place.

An artificial medium can also be used to test the pH response of earthworms. This is something ideally suited to an artificial medium, where there are fewer problems associated with the buffering capacity of the material. It is almost impossible to alter the pH of an organic material without there being a buffering effect and consequent pH drift (Kaplan *et al* 1980).

8.4.2) The Development of a Synthetic Medium.

The earthworms natural environment can be divided into four fundamental components which can be replicated in a synthetic medium by the following; an inorganic nutrient solution, an organic nutrient solution, a cellulose base and a microbial inoculum (a suspension of 19g wet weight solids in 100ml water)

The nutrient solution was made up as follows;

Solution One	Solution Two
100ml distilled water	100ml distilled water
10g NH_4Cl	1g CaCl_2
10g K_2HPO_4	
2g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	
2g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	

The inorganic constituents were chosen to encourage a wide range of aerobic microbial growth as described by Stanier *et al* (1971). General purpose reagents were used to allow the impurities to provide the micro-nutrient requirements of the culture.

Two solutions were prepared to avoid problems of precipitation with calcium salts. Although this has no effect on the nutrient value of the solution, precipitation was found to make taking dilutions of the standard solution difficult.

As an initial trial run three dilutions of the stock standard were taken, 10x, 20x and 100x dilution. These were then added to a powdered cellulose base in the ratio 50ml of nutrient broth to 10g cellulose, with two replicates per dilution. The cellulose and broth were thoroughly mixed and then stored in an incubator at $20 \pm 1^\circ\text{C}$ for two days. After this period four healthy adult *E. fetida* were placed on the surface of the cellulose mix in each treatment and the containers returned to the incubator. At this stage no microbial inoculum was added to the medium. The containers were examined after 2 and 12 hours and any signs of mortality recorded. All earthworms at 10x dilution died, but at 20x and 100x dilution all earthworms survived for 12 hours and beyond, moving into the waste. As cellulose is not toxic to earthworms, mortalities at 10x dilution must have been due to either a general ionic conductivity or pH effect or because of the toxicity of a specific salt, the most likely candidate being the NH_4^+ cation.

In a second trial run a modified dilution range of the stock standard was used as follows;

100x dilution (1ml in 100ml)

33x dilution (3ml in 100ml)

20x dilution (5ml in 100ml)

14.3x dilution (7ml in 100ml)

The nutrient mixes, before addition to cellulose were analysed for pH and conductivity as in section 5.1.3. The results are presented in graph 8.4.2.1 at the end of the section.

The diluted nutrient solutions were added to powdered cellulose in a 50ml to 10g ratio, along with 0.05g lab-lemco and 0.5ml microbial inoculum per container. The mixture was thoroughly mixed and placed in an incubator at 20°C for 48 hours, an adequate period for a mixed cellulose decomposing micro-flora to develop (Morgan pers. comm.).

At the end of this period the containers were each inoculated with a known weight of four earthworms of approximately 0.2g in weight. At this stage there was no visual appearance of microbial colonies developing on the cellulose. The containers were maintained at 20°C and the earthworms handsorted, weighed and returned to the container after 3, 12, 19 and 26 days. The results are presented in graph 8.4.2.2 at the end of the section.

In this trial run weight gains were observed in the media with lower nutrient dilutions, but at 14.3x dilution half the earthworm population died after 3 days, and by 12 days all the earthworms had died.

One possible factor causing earthworm mortalities was pH. The nutrient medium was also observed to have a low pH which tended to rise on dilution of the nutrient solution. The low pH is likely to have derived from the dissociation of some NH_4^+ ions to NH_3 and H^+ ions. K_2HPO_4 will have a limited buffering effect;



Obviously the dissociation of the NH_4^+ ions is greater than the buffering capacity of potassium hydrogen phosphate. On dilution the hydrogen ion concentration is reduced and so the pH falls. Kaplan *et al* (1980) tried with difficulty to alter the pH of sewage sludge to identify its effects on earthworms. The results suggest *E.fetida* can survive a pH range of 5 to 9, with an optimum at 7, although the results here show that earthworms survived in cellulose-mineral nutrient mixes with a pH of approximately 4. Possibly the earthworm response to pH depends on the presence of ions with solubilities which alter with pH, as in aluminium ions and 'acid rain'.

To try and optimise the pH the stock standard was titrated with 0.2M potassium hydroxide. pH 7 was achieved with 38.5ml of 0.2M KOH, equivalent to 0.43g of KOH. A new nutrient solution was therefore made up with the following constituents;

100ml distilled water

1g NH_4Cl

1g K_2HPO_4

0.2g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

0.2g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$

0.43g KOH

The pH of this solution was 6.67 and on the addition of 0.1g CaCl_2 in 100ml distilled water the pH rose to 6.75 and so the pH was finally adjusted to 7 by an addition of 4ml 0.2M KOH.

From this stock solution the following dilutions were taken;

2000x dilution (0.05ml in 100ml)

1000x dilution (0.1ml in 100ml)

500x dilution (0.2ml in 100ml)

333x dilution (0.3ml in 100ml)

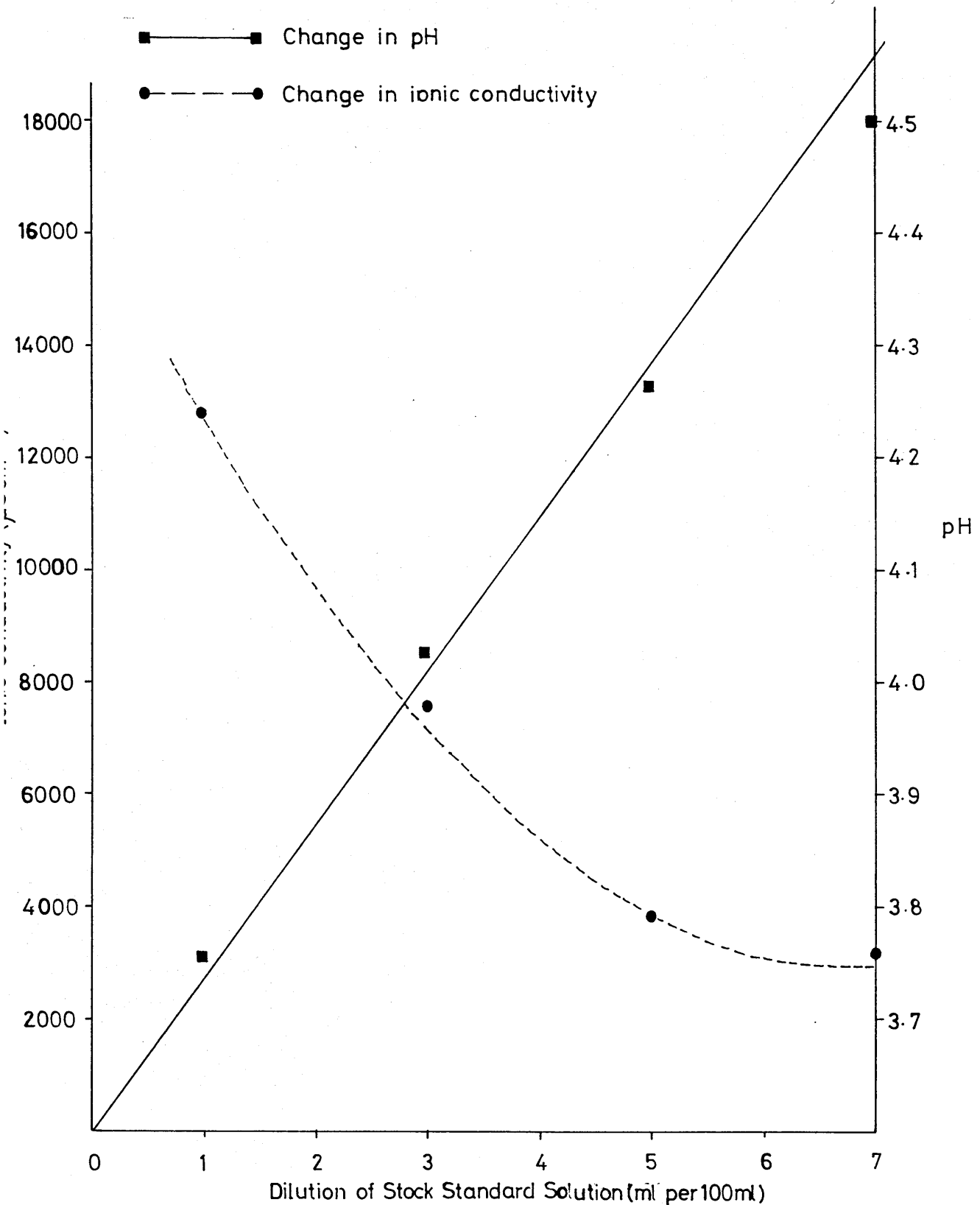
250x dilution (0.4ml in 100ml)

These were added to plastic containers at the rate of 50ml diluted nutrient solution to 10 ml cellulose and 10ml microbial inoculum with four replicates for each treatment. To two of these replicates 'Lab Lemco', a dry powdered organic nutrient derived from beef extracts, was added at the rate of 0.05g per container in order to observe its effects on earthworm growth. The containers were stored in an incubator for a period of 5 days to allow bacterial populations to develop.

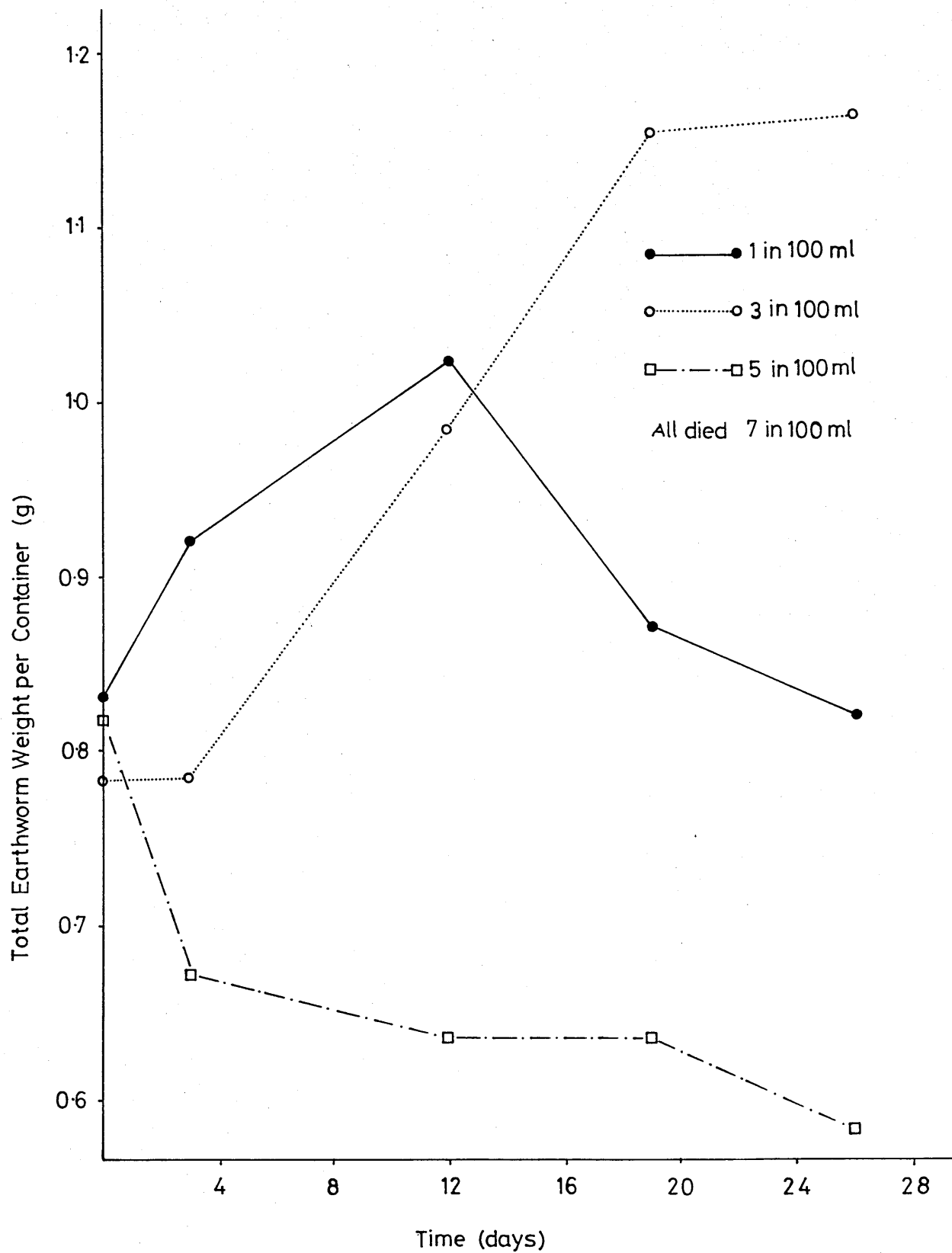
The synthetic media were then inoculated with juvenile *E.fetida*, approximately 0.15g in weight. The normal practice of using hatchlings at 0.02g was not employed here because the potential environmental stress the earthworms may have been subjected to in a synthetic medium would be better tolerated by older earthworms. Obviously a compromise between the age of the earthworms and their capacity for further growth must be met.

The earthworms were weighed weekly after first being washed in distilled water and dried on tissue paper. Before the earthworms were returned to the container the synthetic medium was thoroughly mixed to aerate it and evenly distribute microbial cells. The weight change of the individuals can be seen in graph 8.4.2.3.

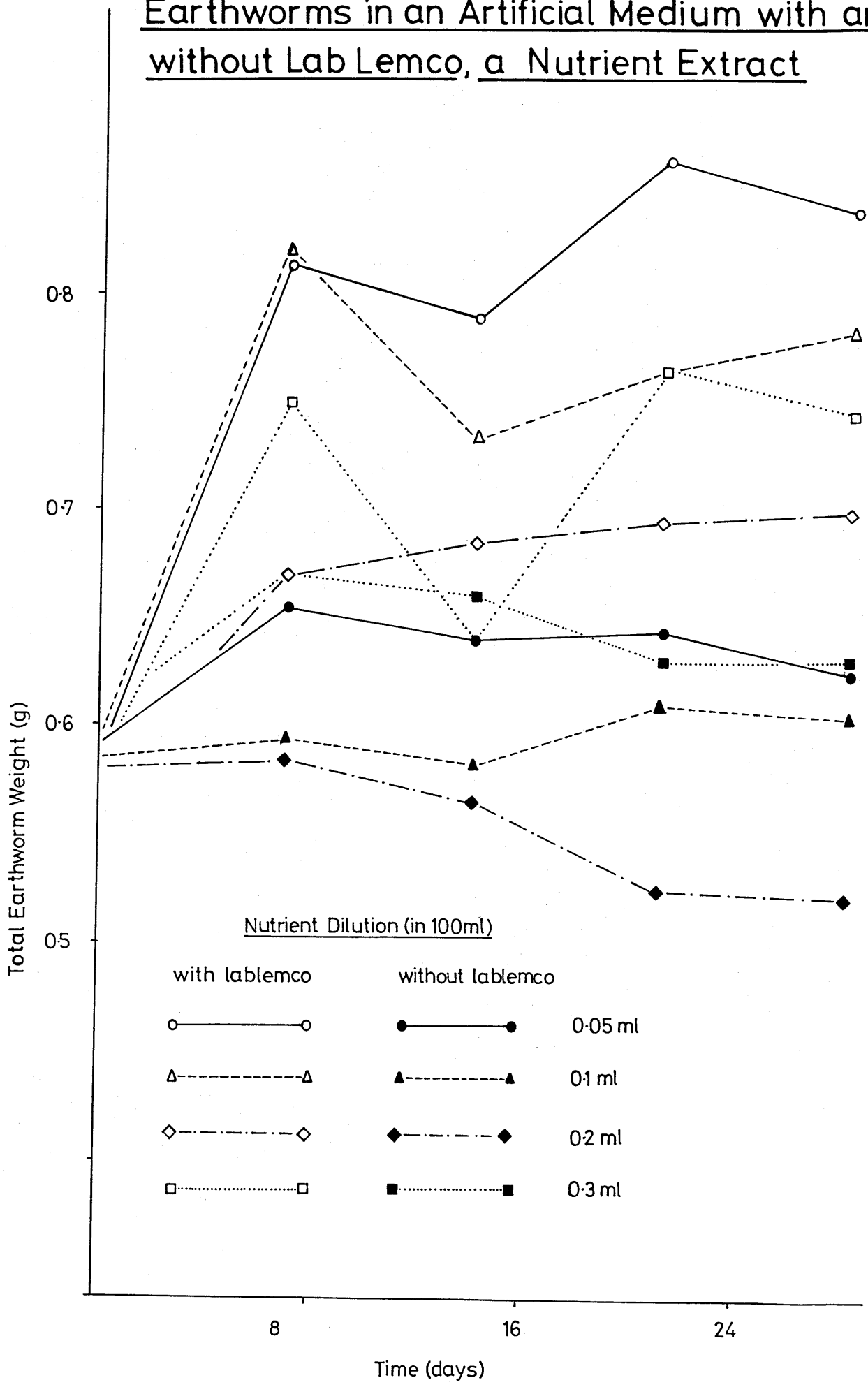
Graph 8.4.2.1. Changes in the pH and Ionic Conductivity on the dilution of a Standard Microbial Nutrient Solution



Graph 84.2.2. The Growth of Four Earthworms
in a Cellulose Substrate with Differing
Dilutions of a Microbial Nutrient Solution



Graph 8.4.2.3. The Growth of Four Earthworms in an Artificial Medium with and without Lab Lemco, a Nutrient Extract



8.4.3) Discussion of Results.

After altering the mineral nutrient mix to take into account the problems of ionic conductivity and pH growth curves were obtained for a range of mineral nutrient extracts, with and without the addition of 'Lab Lemco' extract to provide a source of complex nutrients for microbial activity.

The results show the greatest observed increase in weight is poor compared to similar experimental treatments in cattle solids. The three trials recording the highest weight gains also show quite rapid fluctuations in weight gain and loss, indicating a degree of instability in the system. Other trials showed no weight gain, or a loss in earthworm weight.

The synthetic medium can be seen from these results not to be providing *E.fetida* with the environmental and nutritional conditions it requires for active growth. The possible reasons for this are manifold. Despite attempts to manipulate the pH and ionic conductivity of the medium, some factor within the mineral nutrient solution may have put stress on the earthworm and so reduced the growth rate. An individual ion may have caused toxicity problems even though the final mineral nutrient mix was greatly diluted. The results show that the addition of 0.05g of 'Lab Lemco' to the synthetic medium stimulated earthworm growth, as in its absence earthworms lost or remained the same weight. 'Lab Lemco' could either be essential for microbial development in the cellulose base or conceivably it is being taken in directly by the earthworms as a food source. Certainly the wet weight increase in biomass could be accounted for by the small amount of 'Lab Lemco' added as a dry powder. If this is so then the whole basis of creating a synthetic medium is lost.

By having a single carbon source in the synthetic medium there may have been a trend towards microbial monocultures which eventually toxify the environment with their

metabolic waste products, causing a very fast population decrease followed by a slow increase. This is less likely to occur in organic wastes, as the metabolite of one group of microbes immediately becomes the substrate of another. Fluctuations in microbial population numbers could easily account for earthworm weight losses and gains, especially if they were also affected by the build up of microbial metabolites. Hartenstein (1981) argues that protozoa are essential in the diet of *E.fetida*. If he is correct and such groups of micro-organisms did not develop in the artificial media described in this section this could explain the results obtained.

There was visual evidence that some containers became colonised by the fungal species *Aspergillus fumigatus*. This occurred most in the controls or in treatments where earthworms grew poorly or died. This suggests a form of negative feedback system whereby containers with active earthworm species ingest the cellulose base consuming microbial cells, and mixing and aerating the material, encouraging a more varied microbial growth.

From the results a great deal of further work is required to produce a synthetic medium capable of supporting earthworm growth to the same extent as an organic waste, and the preliminary indications are that the original idea of producing a simplified environment in which to study earthworm growth is untenable. It may be the case that successful earthworm growth can only be achieved in a synthetic medium with chemical composition which is as complicated as the original wastes.

8.4.4) General Problems encountered in Creating a Synthetic Medium for Earthworm Growth.

Although a given medium may be suitable for the initiation of microbial growth, the subsequent development of a bacterial population may be severely limited by chemical

changes brought about by the growth and metabolism of the species themselves. For example cellulolytic microbes may produce organic acids which immediately become the substrate for another group of micro-organisms in organic wastes, but in the microbially simplified environment of a synthetic medium other microbial groups may not exist, causing a build up of secondary metabolites which ultimately reach toxic levels.

Although a mixed microbial culture is added to the synthetic medium, the conditions therein may favour one specific microbial species to develop, as by chance the conditions have been created that are ideal for that organism. Some microbial species may require some growth factor beyond that supplied in the inorganic nutrient base or the 'Lab Lemco' extract. Evidence suggests that *E.fetida* thrives on a mixed microbial diet, and finds mono-cultures unpalatable (Morgan 1986).

If a range of microbes do develop, the diversity still may not be enough to produce maximal growth rates. There is also the possibility that individual earthworms require a period of acclimatisation before achieving acceptable growth and reproductive rates.

E.fetida has evolved over many thousands of years to inhabit and exploit a nutritionally and microbially rich ecological niche, and it may therefore be impossible to create a simplified artificial medium which allows *E.fetida* to act as it does in the environment.

8.5) Discussion and Conclusions.

The effects of manipulating the earthworm organic waste environment can be summarised as follows;

NIRD undigested solids have a lower than optimum C/N ratio of 23 and so added carbon can be effectively utilised by earthworms to increase biomass gains. If separated liquids are used to culture earthworms in conjunction with a bulking agent, chopped straw produces the best earthworm growth response and peat the worse. The results obtained are discussed in terms of the availability of carbon as a nutrient source from the solids, and possible inhibitory effects. A cellulose based artificial medium is not able to support adequate earthworm growth. This is possibly because a medium based on a single carbon source does not allow a well balanced microbial ecosystem to develop. This is essential to provide the environmental and nutritional conditions for earthworm growth.

This work therefore highlights some important features of an organic waste in relation to earthworm growth. Earlier experiments implicated factors such as temperature, ionic conductivity and ammonia ion concentration in producing a non-optimum environment which inhibits earthworm growth. The experiments carried out in this chapter show that if environmental conditions within organic wastes are not limiting, nutritional factors must still be correct to obtain optimum earthworm growth. How much of the carbon present in the wastes is in a form available for breakdown, and the ratio of carbon and nitrogen are the two most important factors.

In animal wastes nitrogen is usually present in excess and processes in the waste usually result in initial N losses through volatilisation, denitrification and leaching. Nitrogen can be stabilised in microbial biomass, but only if sufficient carbon is present in a microbe available form. A high proportion of carbon can be incorporated in or associated with lignin, and so if extra carbon is added in an easily assimilable form it can be utilised

by microbes and thus by earthworms. However, interpretation of results can be difficult because carbon is an integral part of the physical structure of organic wastes as well as providing a primary nutritional source. Therefore as carbon is decomposed the physical composition of the waste can change, altering environmental as well as nutritional factors, highlighting the closely linked nature of these two factors.

The results from this chapter that are most applicable to practical earthworm culture are those relating to bulking agents, showing significant differences between materials. It is in the choice of bedding employed by the farmer for his animals that he can have the greatest influence on the characteristics of the slurry produced, as the bedding makes up a major portion of the fibre in slurry. Patterns of earthworm growth in material from different sites have been attributed to the use of different bedding materials. The results presented here indicate chopped straw is the most suitable carbonaceous bedding material if earthworm culture in the treated material is contemplated. However it should be noted that some farmers employ sand or rubber mats with no bedding, and it is not known what effects this would have on the slurry produced and subsequent earthworm culture.

The choice of bedding material used raises economic questions of whether using more expensive bedding can be offset against increased efficiency of earthworm culture. However, this in itself raises the question of whether waste treatment systems such as earthworm culture should be able to 'bolt on' to existing farm management systems, or whether they can only work if employed as part of an integrated farming system. The second option would give systems such as earthworm culture a better chance of success but would require some wide-reaching changes in farming practice.

Chapter Nine.

Earthworm Induced Changes in Separated Undigested and Digested Solids.

9.1.1) Introduction.

In work from previous sections differences between farm wastes used as a substrate for earthworm growth and the resultant worm worked material have been studied. However these two materials only represent the beginning and end point of a continuous process of change for both physical, chemical and biological factors. Studies of the rate of change of these factors with time with systems with and without earthworms can give valuable information on the actual process of earthworm induced changes, and the feedback between the organic wastes and earthworms. In this section a simple non draining system is set up using multiple replicates of containers with digested and undigested solids to allow for destructive sampling. These are left to decompose over time with and without earthworms, and a proportion of the containers and their contents are taken at set time intervals to provide material for physico-chemical analysis.

There are four possible hypotheses concerning the effects of earthworms on the decomposition of organic materials as measured by changes in physico-chemical analyses; that earthworms have no effect on the decompositional processes within separated solids, or earthworms slow down the rate of decompositional processes, or earthworms accelerate the rate of decompositional processes, or finally earthworms produce conditions in the solids that would not occur in their absence, either by advancing changes beyond their natural end point or initiating new decompositional events that do not occur in their absence.

As discussed in section 1.6, the majority of the changes in physico-chemical factors in organic materials is microbially mediated, and so the hypothetical acceleration or reduction in microbial processes assume an interaction between earthworms and micro-organisms. In the final hypothesis earthworms may stimulate microbial activity which does not occur when earthworms are absent, or may be entirely earthworm induced. To actually separate such responses is very difficult.

9.1.2) Materials and Methods.

Freshly separated digested and undigested cattle solids were collected from NIRD and stored frozen at -12°C . When required approximately 3kg fresh weight of each material was thawed and stored at laboratory temperature (approximately 20°C) for two weeks to undergo mesophilic aging and so become acceptable to earthworms. Once acceptable 60g wet weight of solids were placed in 10cm diameter plastic containers. Digested solids had the highest moisture content at 87.79g, and so the moisture content of undigested solids was adjusted to this figure, rather than try to dry the digested solids to the usual value of 85% moisture content and risk inducing physico-chemical change. 50 containers of solids were prepared for each type of material, and samples of each retained in a refrigerator prior to physico-chemical analysis.

From the 50 replicates of digested and undigested solids, 25 were put to one side as controls, and the others were inoculated with 10 *E.fetida* hatchlings, per container, average weight 0.02g, heat extracted from worked solids. This gave four different treatments; digested and undigested solids with and without earthworms.

Containers with and without earthworms were maintained in incubators at $20 \pm 1^{\circ}\text{C}$ for the duration of the experiment. Once a week the earthworms were handsorted from the solids, washed, dried on absorbent paper towelling and weighed to give an overall earthworm weight per container. At this point the container and contents were also weighed to obtain a weight loss value for the different treatments.

Also on a weekly basis five containers from each of the four treatments were taken at random, associated earthworms removed and the solids from the containers pooled for physico-chemical analysis. Approximately half of the material was stored in its wet state at 4°C , and the other half oven dried, ground to pass through a 1mm sieve and stored in a desiccator prior to physico-chemical analysis. This so-called destructive sampling

regime continued for five weeks until no containers remained.

9.1.3) Physico-Chemical Analyses.

Analyses were carried out as in section 3.1.4 and 5.2.3. Ammonia and ammonium nitrogen was measured by distillation rather than by probe. 5g fresh weight of material was placed in a boiling tube and connected to a 'Buchi' distillation unit. 10ml of 12.5N NaOH is added to convert ammonium ions to free ammonia, and steam passed through the solution. The distillate was collected in a beaker containing 25ml 2% boric acid solution and 3 drops of SHER indicator. The distillate was titrated against 'Analar' 0.1M H_2SO_4 , and the ammonia and ammonium ions present calculated from the formula as in section 3.14.

Potassium and phosphate analyses were carried out on dried ground samples which had undergone dry combustion as described in MAFF (1981).

Fat content of the solids was measured with the 'soxhlet' apparatus. A paper thimble was filled with 1g of dried, ground material and placed in the 'soxhlet' apparatus. Petroleum spirit (b.p. 40-60°C), in a pre-weighed boiling flask with anti-bump granules, is used to extract fats and oils by refluxing at 60°C for 4 hours. When extraction is complete the petroleum spirit is evaporated in a water bath within a fume cupboard. The flask was then dried overnight at 105°C, cooled in a desiccator and weighed.

The Van Soest (1971) forage fibre analysis can be applied to animal manures to give a measure of the type of fibre present. Boiling a sample in neutral detergent solution separates soluble materials such as fats, sugars, starches, pectins, proteins, ammonia, urea and minerals. This leaves hemi-cellulose, cellulose, lignin, fibre bound protein and insoluble ash. A second treatment in boiling acid detergent solution solubilises hemi-cellulose and treating the remainder with 72% sulphuric acid breaks down cellulose. Ashing at 550°C

volatilises the remaining lignin leaving a residue of mainly silica. A modified Van Soest method was adopted as in MAFF (1981).

9.1.4) Calculation of percentage organic carbon.

Given that forage fibre analyses had been carried out on the separated solids it was possible to calculate a figure for the percentage organic carbon present from the values already obtained, based on the weights of atoms from the molecular formulae. Given that cellulose is a polysaccharide based on glucose, with the general formula $C_6H_{12}O_6$, the percentage carbon present can be calculated. Similarly the most common monomer of hemi-cellulose is xylose, $C_5H_{10}O_5$. The percentage carbon in these two molecules can be calculated at less than 40%, because of H_2O loss on polymerisation

Lignin is more difficult to calculate, because it is accepted that lignin is not a molecule with a fixed formula, but rather an umbrella term or several similar molecules which join together in different forms to produce lignin. Four molecules that are known to be precursors of lignin are dehydrodiconiferyl alcohol, D,L-pinorexinol, guaiacylglycerol- β -coniferyl ether and syringaldehyde (Nord and Schubert 1967). The mean carbon content of four lignin precursors can be calculated from their formulae at 64.3%.

Organic carbon associated with nitrogen can be calculated by subtracting the value of NH_3 and NH_4^+ -N from the kjeldahl N to obtain a value for organic N. Calculating a mean C content for the 21 most common amino acids of 48% and an N content of 15% the percentage carbon can be obtained from the value of organic N by a simple calculation. The percentage carbon in fat was calculated from a glycerol and 10 carbon fatty acid at 57.9%.

The calculation of percentage organic carbon allows the earthworm available and non-available carbon to be taken into account, which cannot be done with the

Walkley-Black method of total organic carbon analysis, which is itself prone to errors (see the discussion in Hesse 1971).

The errors in this calculated method are as follows;

Firstly the calculation does not take into account soluble carbohydrates such as mono- and di-saccharides, starch etc, which will be present in the soluble fraction of the Van Soest neutral detergent test. However in a microbially active environment such as separated solids one would expect such molecules to have relatively short half-lives before becoming bound in the microbial biomass in association with N, and so their abundance as a percentage of other forms of carbon is relatively low. The effect on the total organic carbon reading will be to reduce it from its actual value.

Secondly the calculated percentage carbon in lignin, fat and associated with organic N may be prone to errors, as for these three materials no specific molecular pattern holds, but they are made up of groups of molecules that interact differently in varying situations. Also the distinctions between the groups of molecules is not always clear cut. Organic N will not be present exclusively as proteins or amino acids, but also as other molecules such as nucleic acids. The Van Soest lignin value will also include hemi-cellulose and cellulose which is intimately bound with the lignin and so shielded from the detergent and acid attack in the Van Soest method. Similarly some proteinaceous material will be associated with fibre.

In these cases there is no single percentage carbon value that will hold in all conditions, and so inevitably errors in the values will be present. However these errors will affect all samples in the same manner, and so will not change the relationships between the sample values and so still allow valid comparisons.

The calculated values quoted here compare with the generally accepted mean value of

58% for soil organic matter (Hesse 1971), which will be a heterogenous mixture of organic compounds.

9.1.5) Results.

The following graphs show changes in physico-chemical factors over time for digested and undigested solids with and without earthworms;

Graph 9.1.5.1 Volatile solids

Graph 9.1.5.2 pH

Graph 9.1.5.3 Ionic conductivity

Graph 9.1.5.4 NH_3 and $\text{NH}_4^+\text{-N}$

Graph 9.1.5.5 Total kjeldahl nitrogen

Graph 9.1.5.6 Potassium

Graph 9.1.5.7 Phosphorus

Graph 9.1.5.8 Neutral detergent fibre solution

Graph 9.1.5.9 Hemi-cellulose

Graph 9.1.5.10 Cellulose

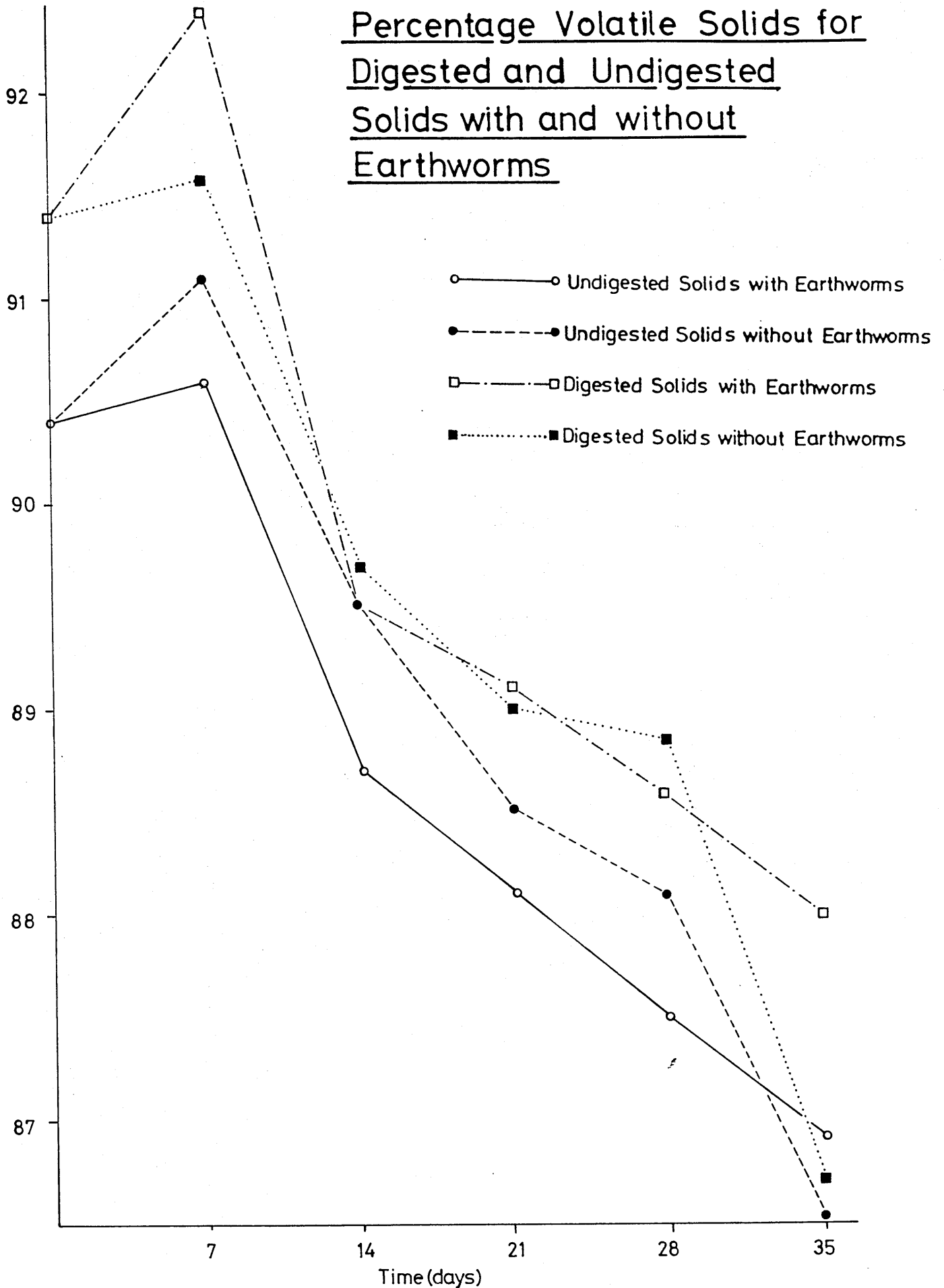
Graph 9.1.5.11 Lignin

Graph 9.1.5.12 Calculated organic carbon

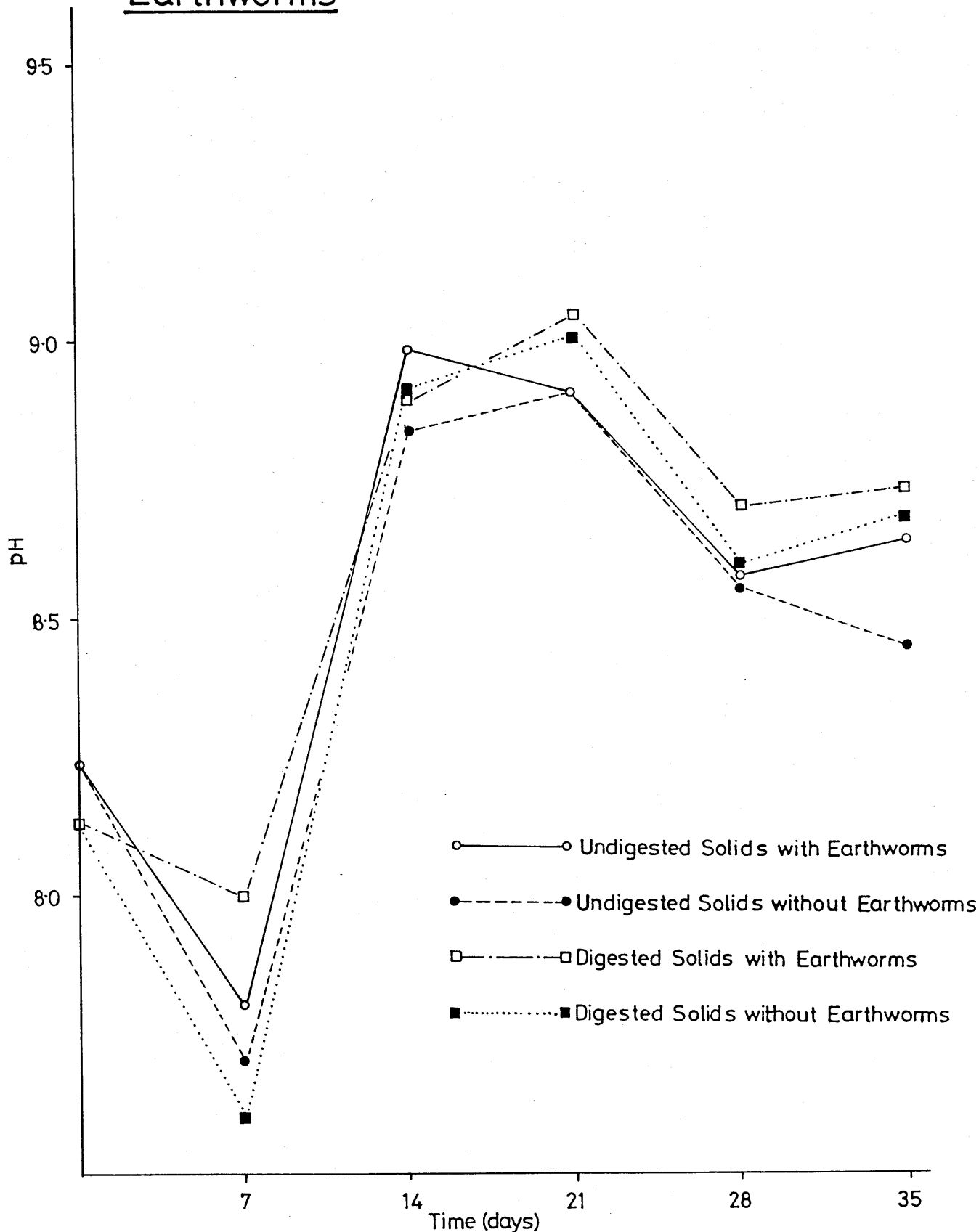
Graph 9.1.5.13 C/N ratio

Graph 9.1.5.14 shows the growth of *E.fetida* in the undigested and digested solids.

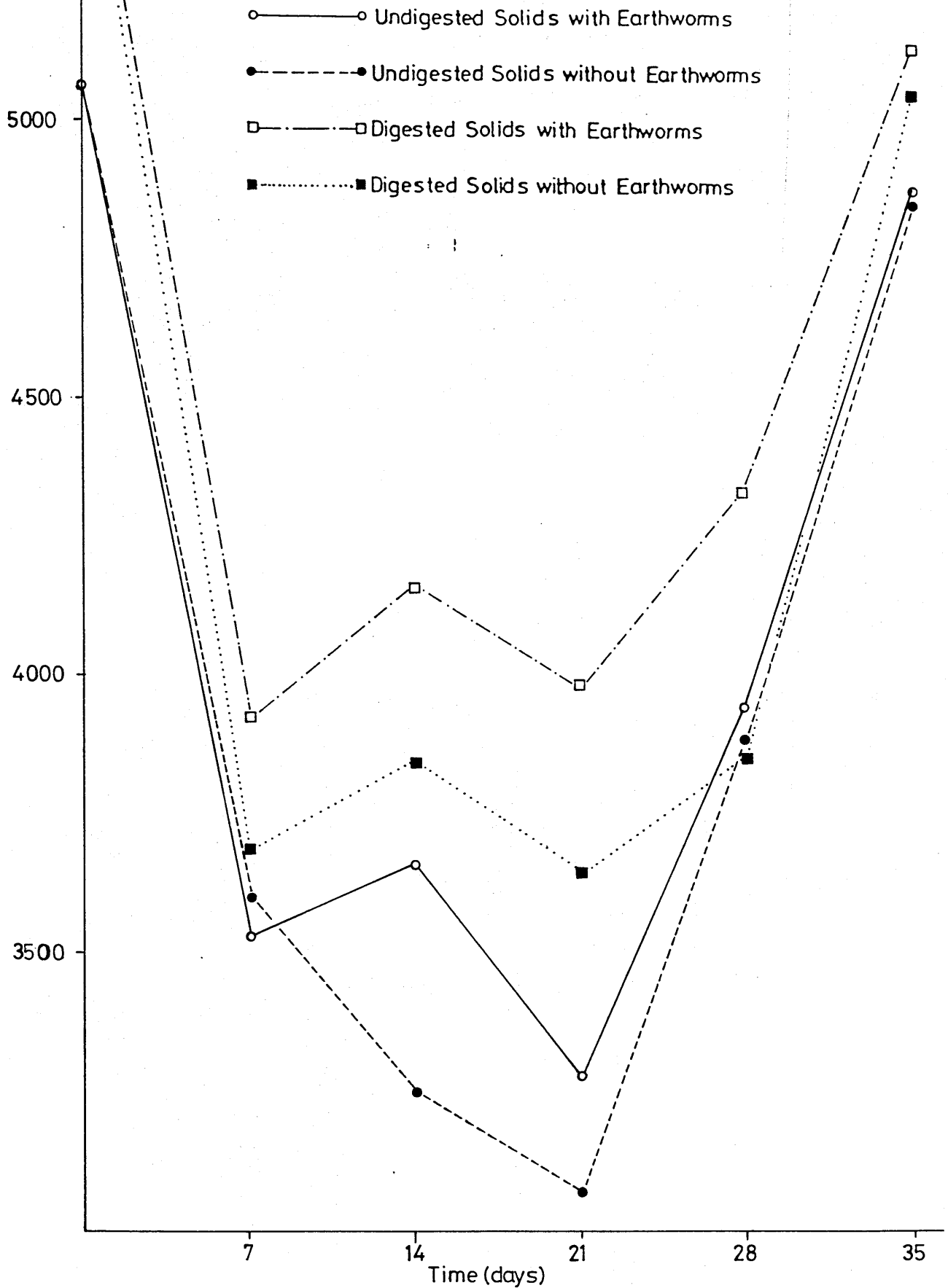
Graph 9.15.1. Change in
Percentage Volatile Solids for
Digested and Undigested
Solids with and without
Earthworms



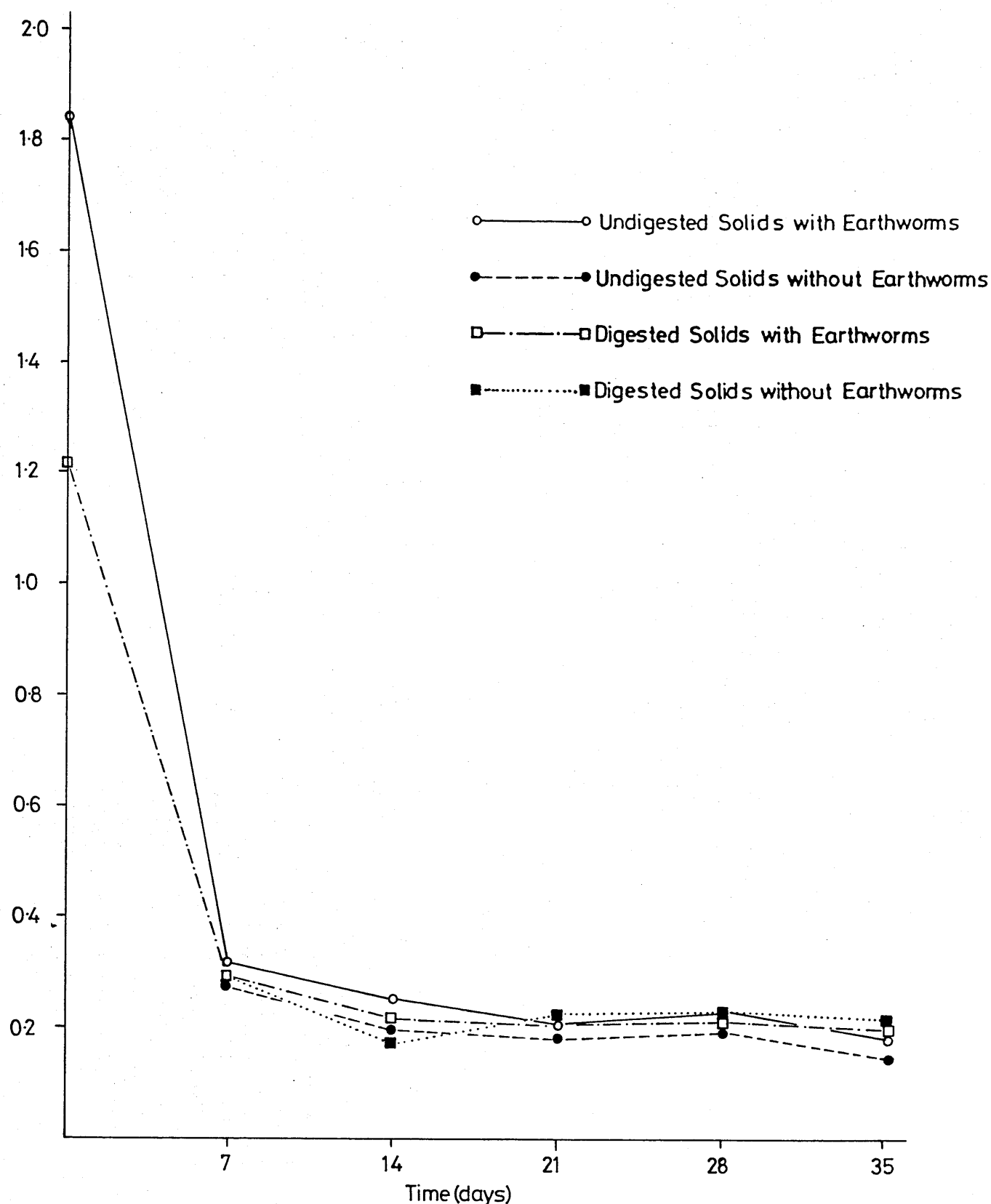
Graph 9.15.2. Change in pH for Digested and Undigested Solids with and without Earthworms



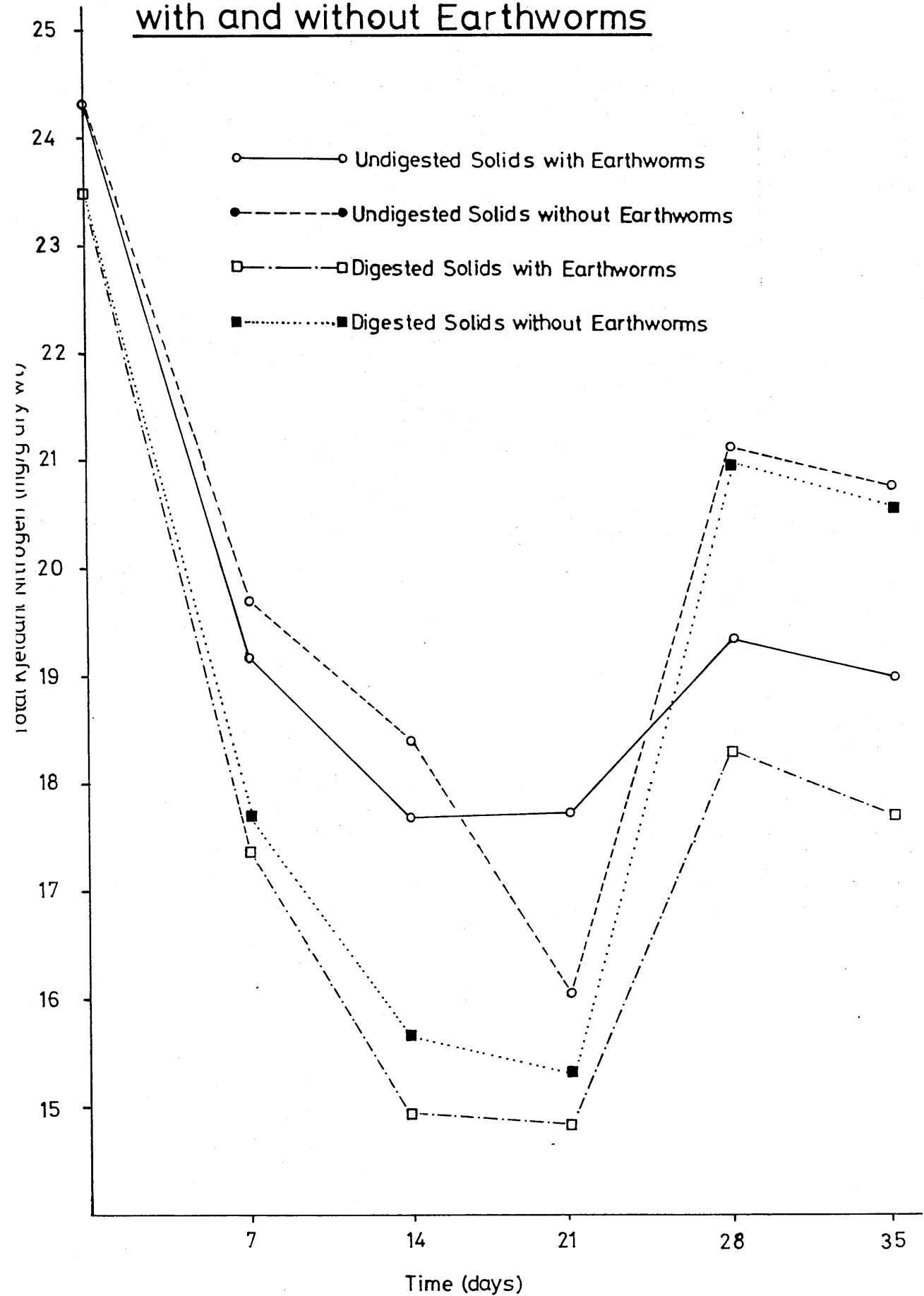
Graph 9.15.3. Change in Ionic Conductivity for Digested and Undigested Solids with and without Earthworms



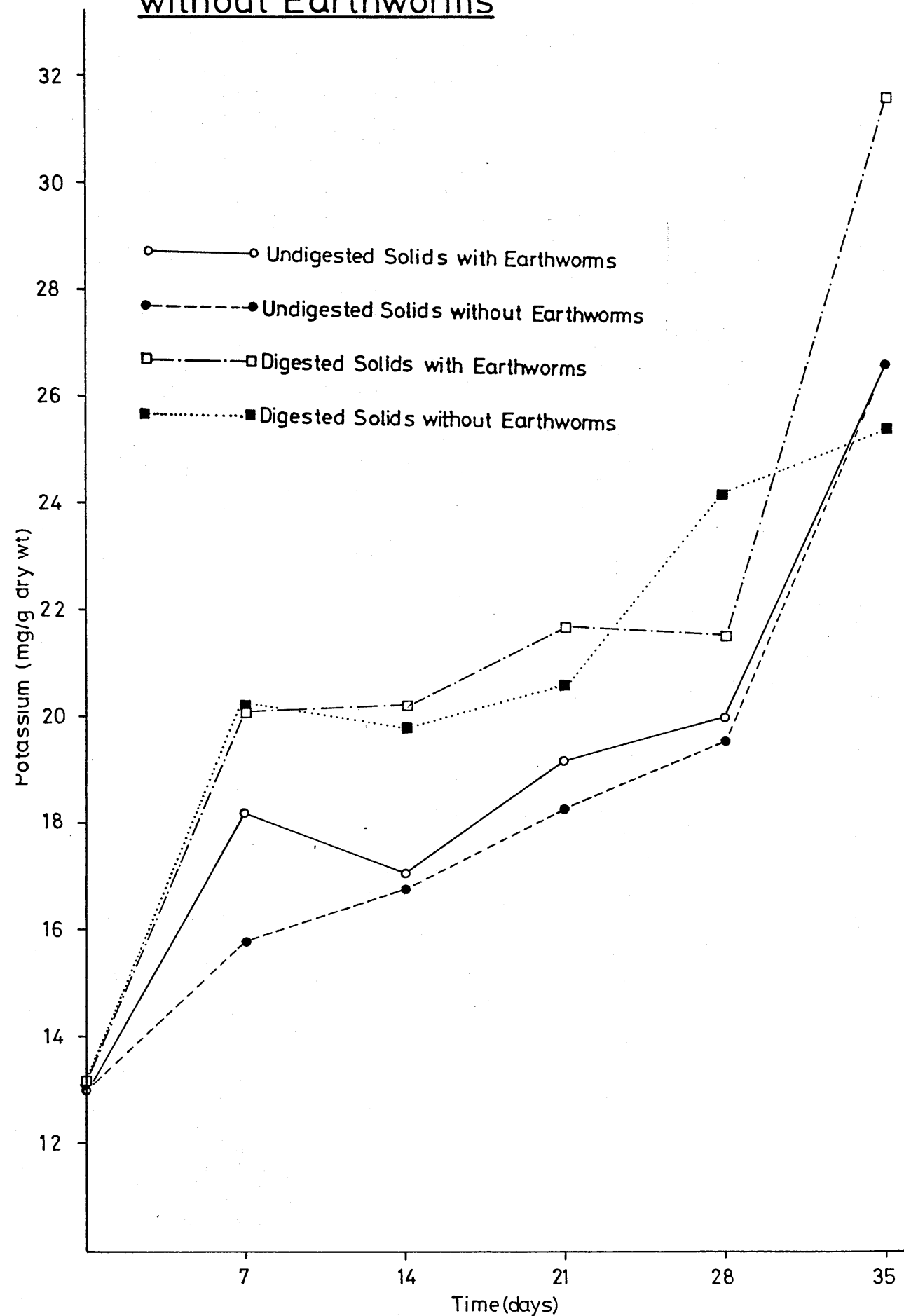
Graph 9.15.4. Change in $\text{NH}_3\text{-N}$ and $\text{NH}_4^+\text{-N}$ for Digested and Undigested Solids with and without Earthworms

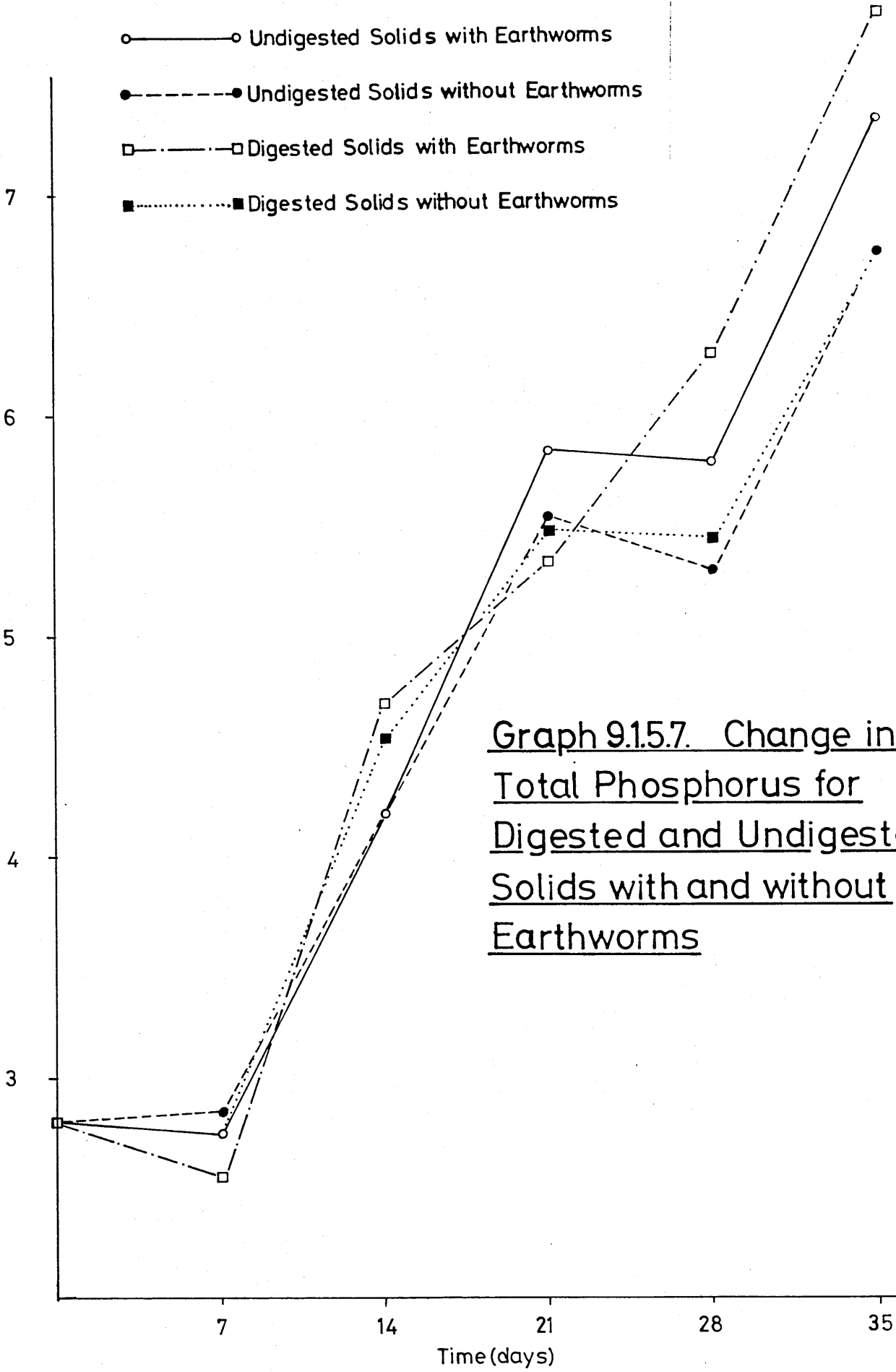


Graph 9.15.5. Change in Total Kjeldahl Nitrogen for Digested and Undigested Solids with and without Earthworms



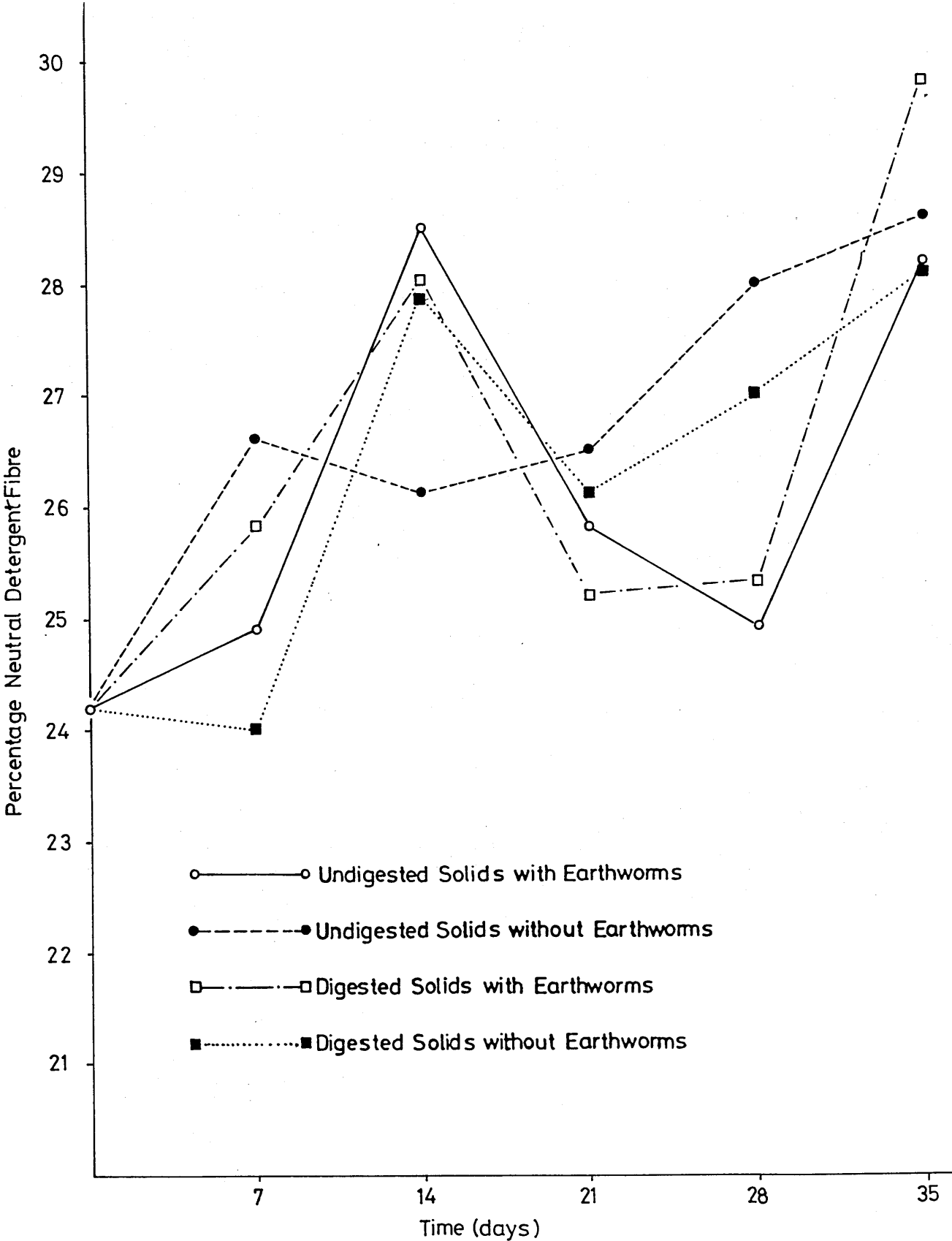
Graph 9.15.6. Change in Potassium for
Digested and Undigested Solids with and
without Earthworms



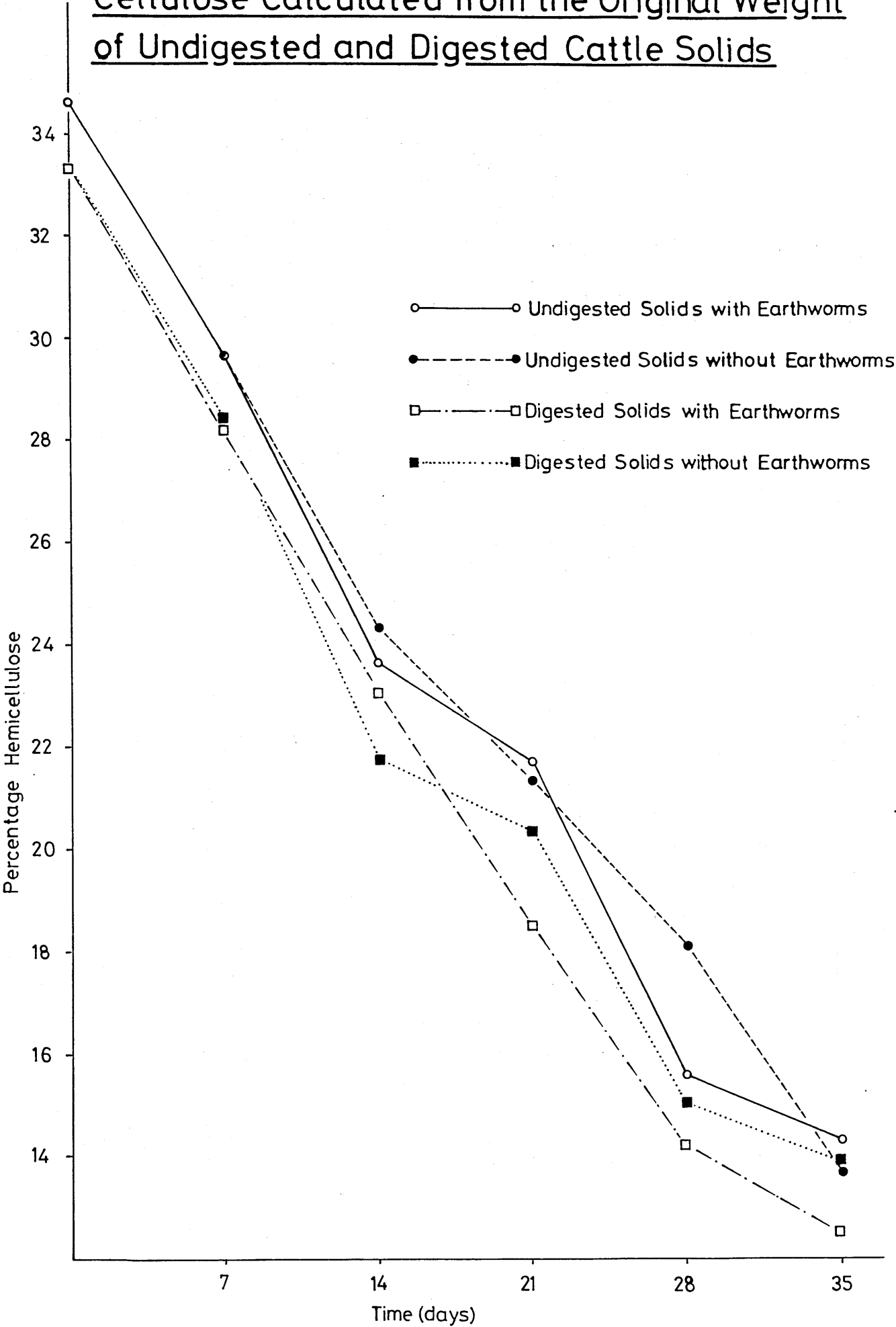


Graph 9.15.7. Change in Total Phosphorus for Digested and Undigested Solids with and without Earthworms

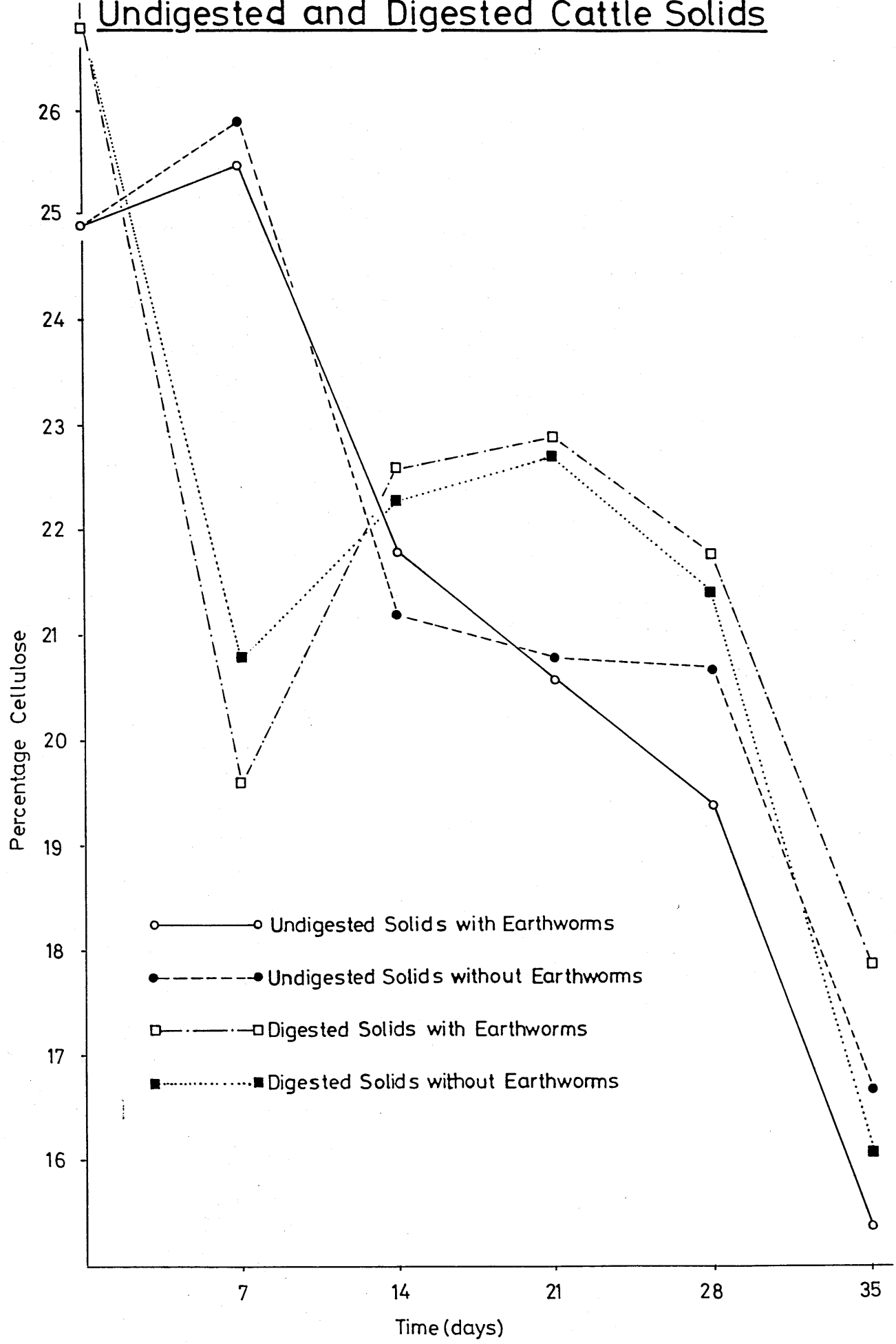
Graph 9.15.8. Change in Percentage Neutral
Detegent Fibre Calculated from the Original
Weight of Undigested and Digested Cattle
Solids



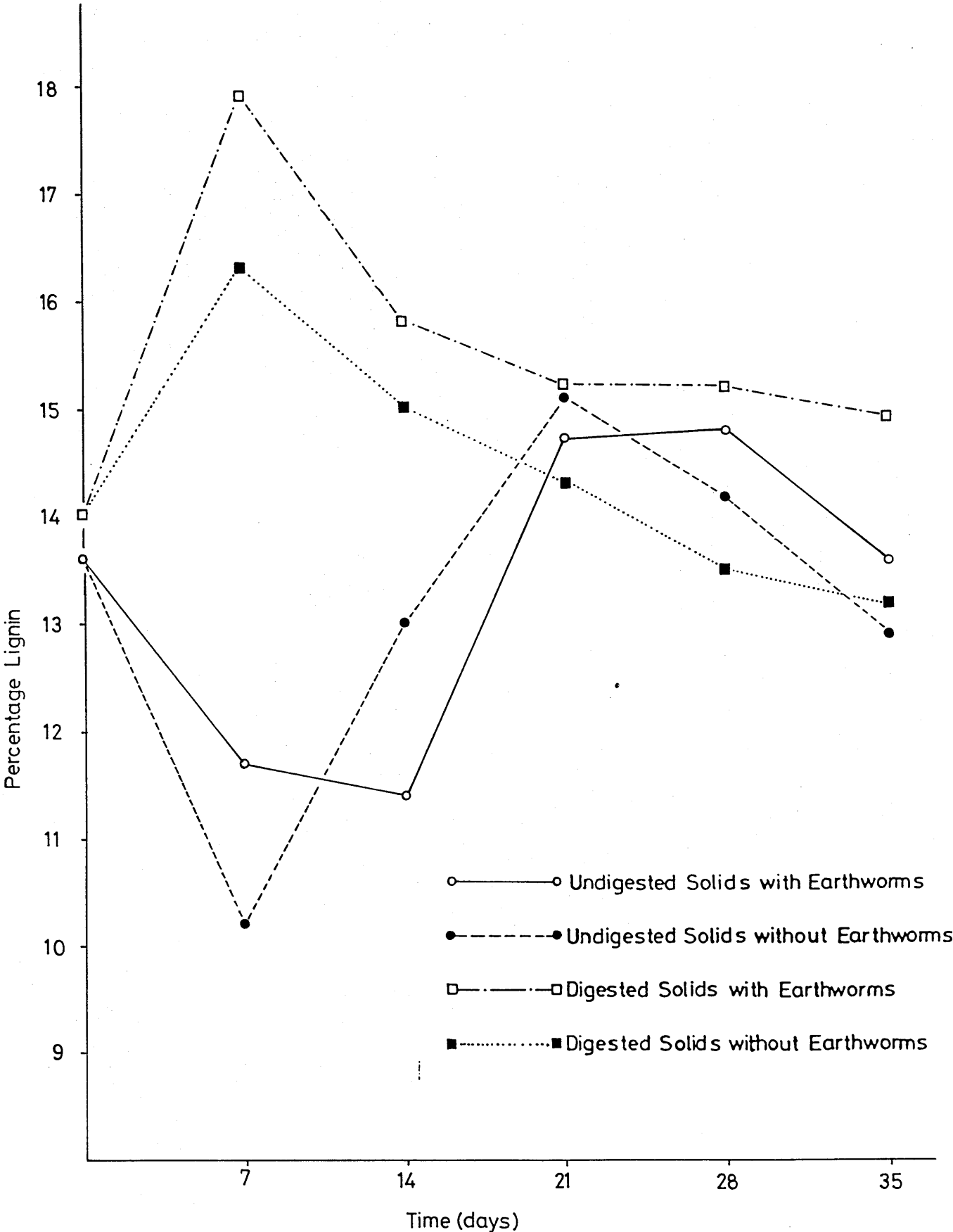
Graph 9.15.9. Change in Percentage Hemi-Cellulose Calculated from the Original Weight of Undigested and Digested Cattle Solids



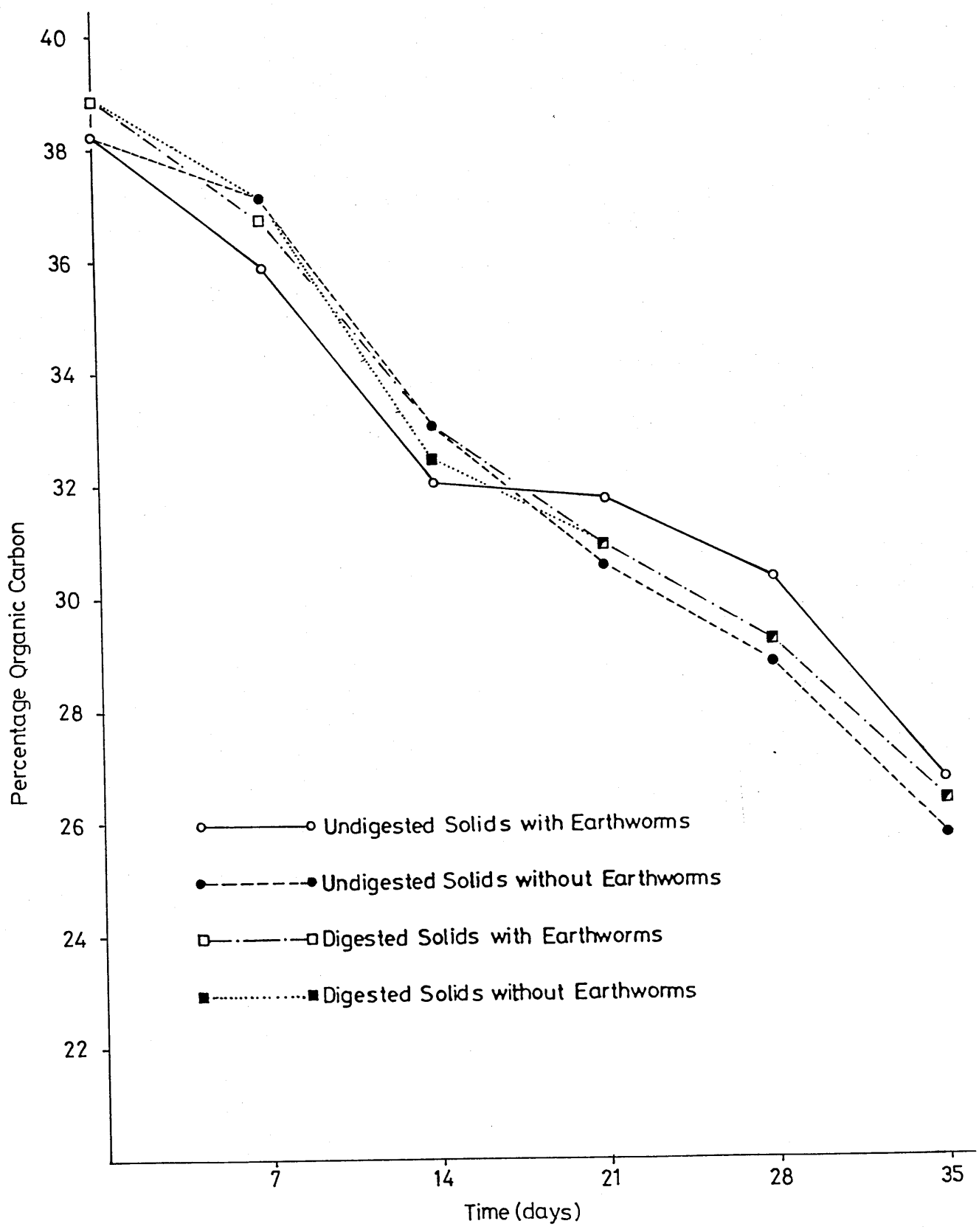
Graph 9.15.10. Change in Percentage Cellulose
Calculated from the Original Weight of
Undigested and Digested Cattle Solids



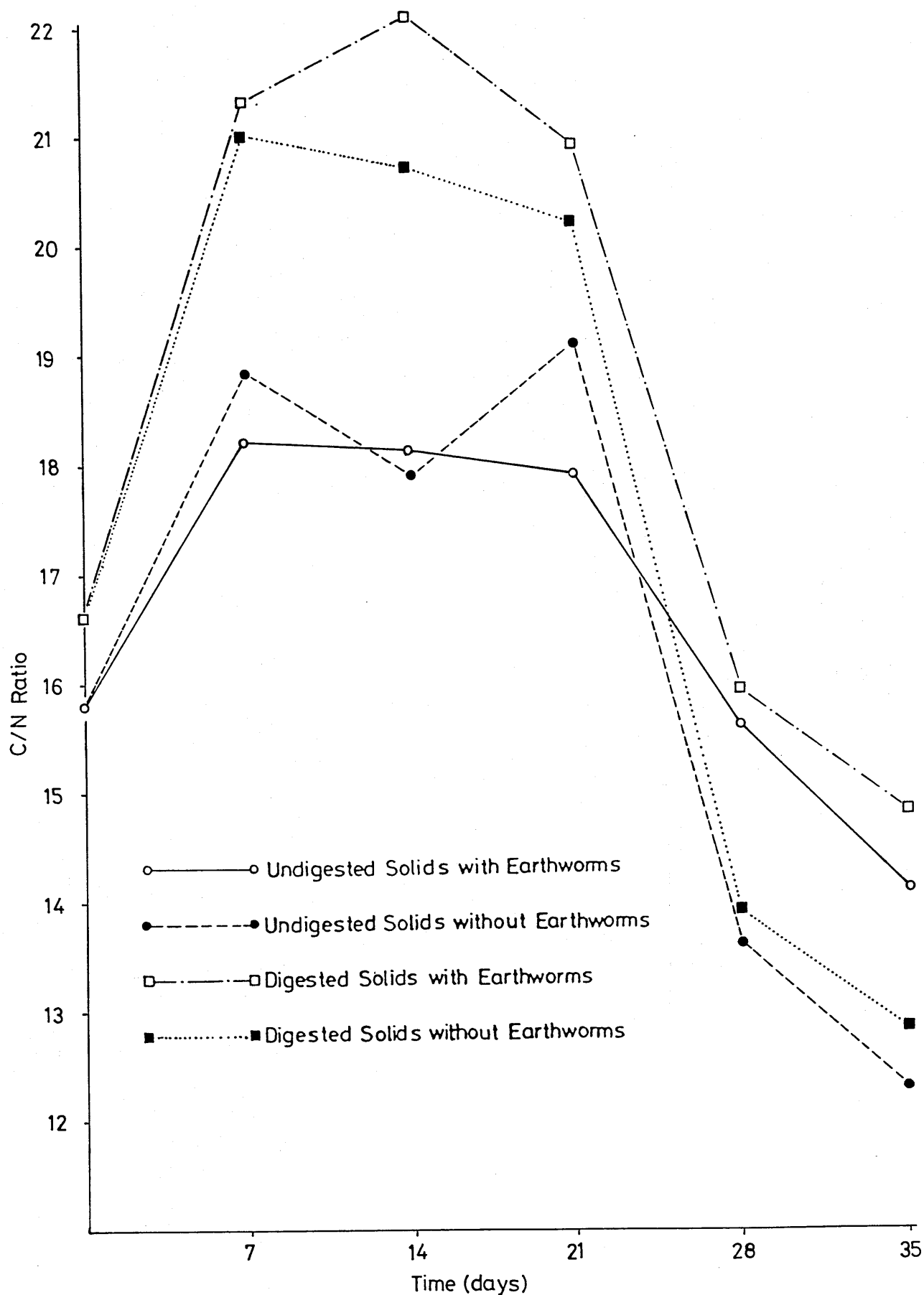
Graph 9.15.11. Change in Percentage Lignin
Calculated from the Original Weight of
Undigested and Digested Cattle Solids



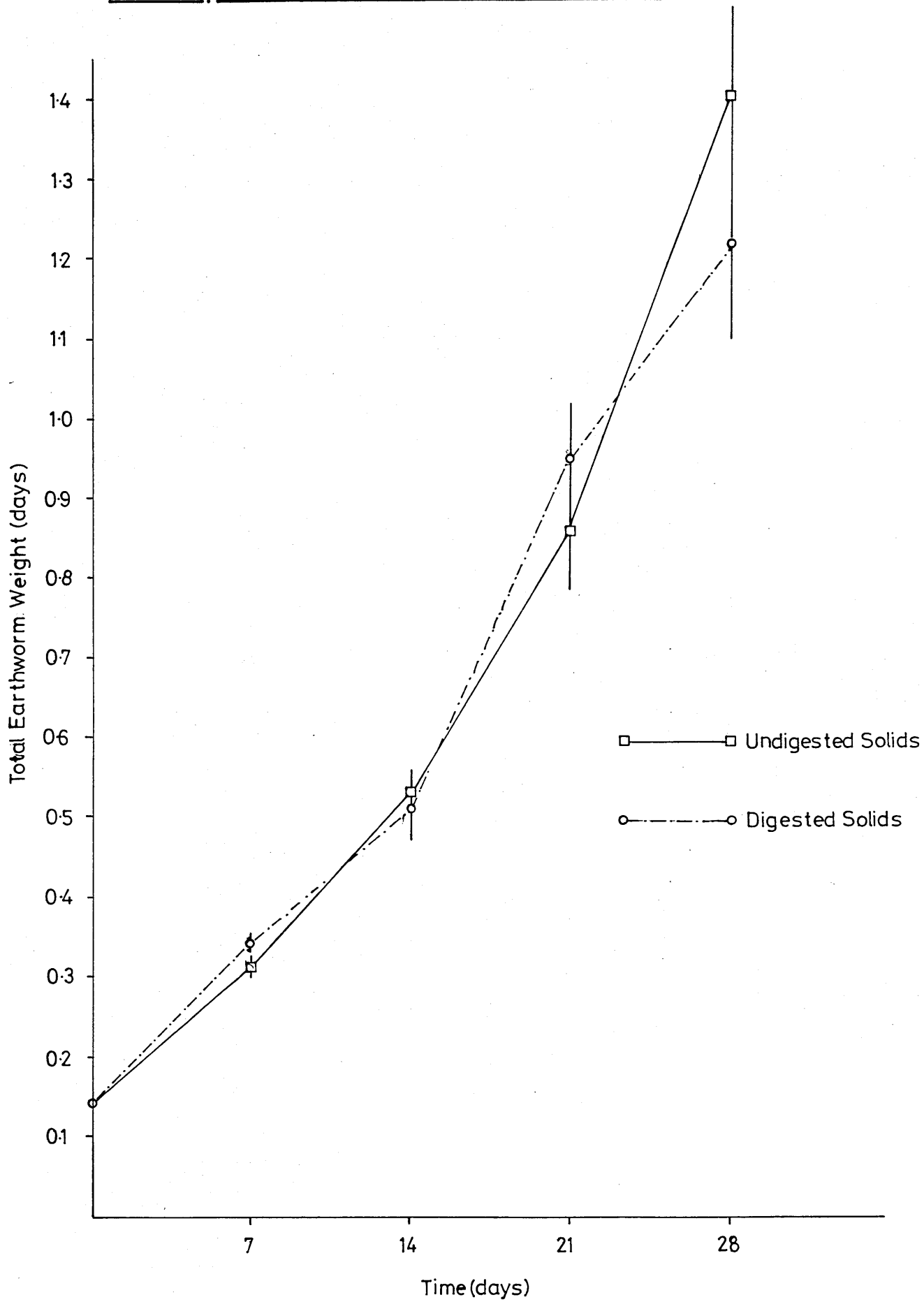
Graph 9.15.12. Change in Percentage Organic Carbon Calculated from the Original Weight of Undigested and Digested Cattle Solids



Graph 9.15.13. Change in C/N Ratio for Digested and Undigested Solids with and without Earthworms



Graph 9.15.14. The Growth of Ten Earthworms
in Separated Solids from NIRD



9.1.6) Discussion of Results.

The changes in each physico-chemical factor will be discussed first, and the results then studied as a whole and the influence of earthworms examined.

Volatile solids.

From graph 9.1.5.1 a small initial increase can be seen for each treatment from week 0 to week 1, followed by a steady decrease for all treatments. Undigested solids have lower values, but all treatments decrease at the same rate. The loss can be explained by the loss of carbon as CO_2 on aerobic decomposition and the incorporation of organic materials into the earthworm biomass. The breakdown of organic N to ammonia which is lost through volatilisation may also add to the loss of volatile solids, although this particular process will be balanced by the microbial incorporation of N into organic materials.

If earthworms are increasing the rate of decomposition one would expect greater volatile solids loss when earthworms are present. From the graphs there is no evidence of this happening, in fact weeks 4 to 5 indicate the opposite trend.

pH

Graph 9.1.5.2 shows the value for pH fluctuating for all treatments, although the changes are not great (less than 1.5 units on the pH scale). All treatments remain on the alkaline side of neutral throughout the 5 week trial.

The reasons for acidification, as seen for weeks 0 to 1 include the volatilisation of NH_3 , the formation of organic acids as a result of decomposition, and the breakdown of fats and oils to release volatile fatty acids (the last two processes usually occurring in anaerobic

conditions).

Increases in the pH as seen between weeks 1 and 2 can be accounted for by the breakdown of organic N to ammonia and its subsequent dissociation to an ionic form, and the aerobic decomposition of organic and volatile fatty acids.

From the graph there is no difference between treatments with and without earthworms. Because so many factors effecting the pH one would not necessarily expect there to be different pH trends between the treatments.

Ionic Conductivity.

From graph 9.1.5.3 an interesting pattern of ionic conductivity change over time emerges. Between weeks 0 and 1 there is a sharp drop in conductivity followed by large differences between the treatments for weeks 1 to 3, and then a large increase in conductivity values for all treatments.

Between weeks 1 and 3 the ionic conductivity of digested solids was higher than undigested solids, and treatments with earthworms have higher conductivity than those without.

The initial drop of conductivity can be tied in with the loss of $\text{NH}_4^+\text{-N}$ ions from the solids (see graph 9.1.5.4), and the subsequent increase can be linked with a loss of volatile solids causing a relative increase in mineral ions. This can be seen directly for potassium ions and total phosphate (graphs 9.1.5.6 and 9.1.5.7).

Between weeks 1 and 3 these two opposite processes are in balance, and differences between the treatments emerge. Digested solids can be expected to have a higher ionic conductivity because of the loss of volatile solids already experienced on anaerobic digestion.

Ammonia and Ammonium Ions.

From graph 9.1.5.4 the following pattern emerges; Undigested solid has an initially higher NH_3 and NH_4^+ ion concentration than digested solids. Between weeks 0 and 1 there is a large decrease to a low level, and between weeks 1 and 5 the values for all treatments stays at a low, steady value of 2-3mg/g wet weight through the process of volatilisation.

Total Kjeldahl Nitrogen.

From graph 9.1.5.5 there is a fall in total kjeldahl nitrogen (kjN) for all treatments between weeks 0 and 2. Between weeks 2 and 3 the values are stable, except for undigested solids without earthworms which continues to fall. Between weeks 3 and 4 there is an increase in total KjN for all treatments, and then a slight decrease during the final week of the experiment. Undigested solids has an initially higher total KjN. The observed decrease in total KjN can be explained by the mineralisation of organic N to NH_3 and its subsequent volatilisation. In treatments with earthworms there will be a net loss of N in the earthworm biomass which is not included in the total KjN value.

The increase between weeks 3 and 4 is more difficult to explain. As with ionic conductivity it may be a relative increase in concentration which accompanies the loss of volatile solids through aerobic decomposition.

If earthworms are stimulating N mineralization and incorporating organic N into their biomass, one would expect that treatments with earthworms present would show the greatest loss of total KjN. This is not clear in weeks 0 to 4, but in week 5 both treatments with earthworms have lower values. It is at the end of the experiment when the greatest earthworm stimulated differences should be apparent, because at this point the maximum amount of separated solids will have been influenced by the activity of earthworms in the containers.

Potassium and Phosphorus.

Graphs 9.1.5.6 and 7 show a steady increase in total potassium and phosphate levels over the 5 week period of the experiment. The observed increases are only relative because of volatile solid losses. For potassium there are generally higher levels in digested solids for the same reasons as the higher recorded conductivity levels. This is not the case for phosphorus because a proportion of the element is in the organic form.

There is no evidence of earthworm stimulated increases in the relative concentrations of these elements by accelerating the rate of aerobic decomposition.

9.2) Van Soest Fibre Analysis.

9.2.1) Introduction.

The graphs of percentage neutral detergent fibre, hemicellulose, cellulose, organic carbon and lignin relate to the relative proportions of these materials in the original cattle solids at day zero. This removes the problem of relating a percentage change to an overall total which is also changing because of decompositional processes occurring within the solids which can be seen from graphs 9.1.5.15 and 16 showing wet weight losses. The T.S. values measured each week show only a small proportion of this weight loss is as water vapour, the rest being made up of CO_2 and NH_3 as it was a non-draining system.

9.2.2) Neutral Detergent Fibre Solution.

From graph 9.1.5.8 a general increase in the amount of neutral detergent fibre (NDF) soluble material for all treatments can be seen. This occurs as non-soluble materials are converted to a soluble form, such as the breakdown of organic P and N and polysaccharides such as hemi-cellulose and cellulose. There is no evidence from the results of an increase in the formation of NDF soluble material by earthworms.

9.2.3) Hemicellulose.

During the course of the experiment there is a large, steady fall in the relative amount of hemi-cellulose for treatments. Hemi-cellulose must therefore be an easily degradable. At the end of the experiment there is a suggestion from graph 9.1.5.9 that the relative loss of hemicellulose is greatest from digested solids with earthworms. This may be a combined effect of hemicellulose loss on anaerobic digestion, and earthworm stimulated microbial activity.

9.2.4) Cellulose.

There is an overall drop in cellulose from its value in the original material, as one would expect from the action of aerobic micro-organisms possessing cellulase enzymes. Unfortunately differences between treatments are difficult to draw because of the obvious artefact of the digested solids value on day 7, showing a large decrease followed by an increase in cellulose that is impossible to explain in the context of these pot experiments. The results are mirrored in graph 9.1.5.11 for lignin, again an obvious artefact. That the rest of the data points for digested solids follow the general pattern of cellulose loss set by undigested solids supports the indication that the values for day seven are aberrant. It is difficult to explain why this has occurred, but could reflect the non-continuous nature of destructive sampling.

In theory one would expect that in passing through a digester anaerobic cellulolytic bacteria will have initiated the breakdown of hemi-cellulose and cellulose. As shown by Hobson (1985), the most accessible and easily degradable fibre will have been broken down initially, indicating that on discharge and separation digested solids will have less cellulose and hemi-cellulose that is liable for breakdown, and a higher proportion that is bound to lignin and therefore less degradable. However, to counter this trend the anaerobic bacteria in the digester will also expose fresh areas of fibre for subsequent microbial degradation.

There may be changes in the availability of cellulose from the fibre over time, as easily degradable material is broken down, requiring the degradation of more resistant C to expose new cellulose sites. There is not likely to be any breakdown of lignin over this period. In experiments in mushroom composting Waksman and McGrath (1931) found that lignin was not utilised after 47 days of composting before spawning.

The results indicate the importance of the physical relationship between the three main

constituents of fibre, hemi-cellulose, cellulose and lignin on its decomposition. Although there is ample evidence that earthworms have associated cellulase enzymes (Parle 1963a), and the macerating action of the earthworm gizzard may expose new sites of hemi-cellulose and cellulose to microbial breakdown, there is no increase in the rate of cellulose breakdown in containers with earthworms.

9.2.5) Calculated percentage organic carbon.

There is a reduction in organic carbon over time, mainly as CO_2 released through aerobic metabolic processes on decomposition. It is important to note that at time zero, 22.9% of the total percentage carbon in undigested solids, and 23.1% of total percentage carbon in digested solids is within lignin molecules, and therefore not available for breakdown. As discussed, the lignin will also shield a proportion of cellulose and hemicellulose from degradation, although this may be taken into account in the lignin value produced by the Van Soest method.

The incorporation into microbial biomass will stabilise some carbon although in contrast incorporation of carbon into earthworm biomass will contribute towards carbon loss because earthworms are separated from the solids before physico-chemical analysis. For undigested solids the maximum recorded earthworm biomass was 1.4g wet weight, or 0.25g dry weight (assuming earthworms are 18% dry matter, Hartenstein 1981). With 60% of earthworm tissue as carbon, this gives a value of 0.15g earthworm incorporated carbon. Even taking this into account there is no evidence of increased carbon losses brought about by earthworm activity.

9.2.6) Change in C/N ratio.

Graph 9.1.5.13. shows an initial increase in C/N ratio for all treatments, and then a sharp reduction below the initial value. The increases for digested solids are higher than

those of undigested material.

Because the containers represent non-draining systems the only loss of carbon and nitrogen can be as the gases CO_2 , NH_3 or N_2 if denitrification is occurring, and also as part of the earthworm biomass.

From the pattern of graph 9.1.5.13. the decomposition process over the 35 day period examined can therefore be divided into three phases: at first nitrogen losses are greater than carbon, fuelled by the high initial volatilisation losses recorded by graph 9.1.5.4. A plateau is then reached with no relative carbon and nitrogen losses, with values close to the optimum for decomposition quoted by Gotaas (1956). Finally there is a fall in C/N ratio, representing a relatively greater loss of carbon. This could occur if most available nitrogen became microbially bound, and competition for carbon as a metabolic resource occurred.

9.3) Calculation of Earthworm Gut Throughput.

From the graphs of physico-chemical changes over time for digested and undigested solids there is no evidence of earthworm influence on the results, although there was an actively growing population within the containers, moving through and feeding on the separated solids. The actual amount of material consumed can be theoretically calculated as follows;

Assume an *E.fetida* gut load measured in horse manure or sewage sludge at 10% dry weight/dry weight, and an average transit time of 2.5 hours (Hartenstein *et al* 1981).

Therefore in one day *E.fetida* consumes $24/2.5 \times 10\%$ of body weight

= 0.96g dry weight/g dry weight of earthworm per day

Assume the dry matter content of *E.fetida* is 18% (Hartenstein *et al* 1981),

and the mean dry matter content of material in this experiment is 13%.

Then 0.96g/g dry weight per day

= $7.38/5.55$ wet weight solids to wet weight earthworms

= 1.33g wet weight/g wet weight per day.

The mean weight of earthworms taken over the duration of the experiment

= 0.64g

Therefore 0.64g earthworms consumes 0.64×1.33

= 0.85g solids per day

= 29.79g over the 5 week period of the experiment

Theoretically half the material in the solids was passed directly through the gut of the earthworms in this trial, and certainly a visual inspection of the containers showed large numbers of distinctive earthworm casts, although this was a qualitative observation. In moving through the solids, releasing nitrogenous excretions and smearing and mixing casts with non-ingested material, there is no doubt that the earthworms present would have effected a very high proportion of all the material in the container. The question is whether through this contact the earthworms have actually stimulated or depressed the

rate of decomposition, or initiated new forms of decomposition.

9.4) General Discussion.

From the results obtained it appears that earthworms have had no effect, either positive or negative on the change in physico-chemical factors over time. It is possible that earthworms did induce physico-chemical changes that were not recorded for several possible reasons. The five week period of the experiment may not have been long enough for earthworm induced changes to become apparent. The 10 individuals in each container were still actively growing at the end of the experiment and had theoretically consumed only 50% of the material. If enough replicates were available it would be better to continue physico-chemical sampling until the peak earthworm weight was achieved and the population went into decline.

Significant differences may have occurred at microsites within the solids, i.e. within earthworm casts or at aerobic/anaerobic interfaces, but when sampled and analysed as a whole such changes were lost. Baath *et al* (1981) examined the growth of pine seedlings in soil and organic matter from field plantations of *Pinus silvestris* in relation to the presence of various types of soil fauna. The workers concluded that the fauna did not play an important part in nutrient return in the field. However, in reviewing nutrient strategies in soil systems Coleman *et al* (1983) argue that changes at localised micro-sites might be important, and state 'attention to this sort of spacial heterogeneity could lead to new insights in subsequent studies in soil ecology'. This concept is relevant to waste decomposition too.

The general role of earthworms in enhancing mineralization in soils is well known (see review in Edwards and Lofty 1977). *E.fetida* excretes NH_3 or urea depending on its physiological state (Needham 1957), and earthworms enhance the mineralization of organic phosphate (Satchell and Martin 1984). The results show that while ionic conductivity is higher initially in containers with earthworms, these differences are lost by the end of the experiment. One can hypothesise that earthworms accelerate mineralization initially,

but general microbial activity cancels this out at the end of the experiment. High ionic conductivity levels can inhibit earthworm growth (see section 1.5.3), introducing the possibility of a negative feedback system operating. However from the earthworm growth curves (graph 9.1.5.15) no evidence of inhibition is apparent.

If it is assumed that there have not been earthworm mediated changes that have not been picked up by the experimental method, then there are several possible explanations why earthworms have had no influence. From foregoing sections it is generally accepted that earthworms act through the stimulation of micro-organism activity. If some environmental conditions within the containers that earthworms had an influence over were already at an optimum for maximum microbial activity, then further earthworm stimulation could not take place. The containers were certainly manipulated for two environmental earthworm optima, the temperature and moisture content of the solids.

The laboratory experimental situation may also not be relevant to the field scale. The small amount of organic material in each container has a large surface area to volume ratio compared with a large amount of solids in the field. If a major role of earthworms is to increase aeration and so stimulate metabolic activity, this is more likely to occur in a field scale than in small amounts of solids in the laboratory.

Another explanation is that other factors within the waste may also stimulate microbial activity to the same or greater extent than *E. fetida*. For example in soil ecosystems both amoeboid protozoa and a bacterial feeding nematode increased CO₂ output and N and P mineralization with glucose as a carbon source (Woods *et al* 1978). Radioisotope studies showed increased substrate utilisation and nitrogen mineralization in nematode grazed microbial populations, and therefore a higher metabolic activity despite lower numbers of organisms (Anderson *et al* 1981). When protozoa and nematodes were both present respiration was found to be significantly greater (Coleman *et al* 1978).

Although these observations were made in soils the same processes occur in wholly organic materials where amoeboid protozoa and nematodes are likely to be present (Morgan 1986). One could therefore speculate that stimulation of microbially induced decomposition by interactions with other micro- and meso-fauna may also be occurring through the activity of protozoa and nematodes, and that the presence of earthworms is having no extra effect. However, against this argument is the above observation that increasing the complexity of a decompositional system increases its overall activity. Another macro-invertebrate, *Oniscus asellus* has been shown to stimulate both microbial and nematode activity in sewage sludge (Brown *et al* 1978).

9.5) Ideas for Further Work.

This experiment has posed more questions than it has answered. Although no direct influence of earthworms on the rate and form of decomposition in this study, it is obvious from the literature that interactions between the waste, micro-organisms and macro-organisms are occurring, and require further study to understand how each group contribute to the observed physico-chemical changes on decomposition. The design of this experiment could be improved by concentrating on one form of separated solids, having more replicates to allow the experiment to continue over a longer time period, and allow a greater number of physico-chemical analyses to be carried out, to include the measurement of organic phosphorus, CO_2 evolution and develop methods of microsite analysis, i.e. possibly harvest and analyse discrete earthworm casts and compare with unconsumed solids. In the longer term the research could be expanded to the field scale, perhaps by containing small amounts of solids in earthworm proof nylon mesh bags which are placed within the bulk of separated solids, and also to initiate microbiological tests, concentrating on cellulose decomposers and nitrogen fixing microbes, if present.

Chapter ten.

The Production of Worm Worked Material from Cattle Solids and its Use as a Horticultural Growing Medium.

10.1) Introduction.

The end products of introducing earthworms into organic wastes is an increased earthworm biomass and a worm worked material gaining certain properties on passing through the earthworm gut. If earthworm culture is to be a viable biological process used in the management of organic wastes these two end products must have some value. Recent economic reports (Foote and Fieldson 1984, Fieldson 1984) indicate that the value gained from the worm worked material is of more importance than that which could be gained from the earthworm biomass.

The potential of earthworm worked material can be realised if it can be shown to be capable of being used as a plant growth medium, and this be used as a product in horticulture. The criteria such a material must satisfy are as follows; it should have no malodours, have plant nutrients and micronutrients in the correct form and ratio, have a high moisture holding capacity, have a good aggregate water stability and free of plant and animal pathogens, heavy metals, pesticides or hormonal residues.

The work of this chapter is therefore concerned with evaluating earthworm worked material as a plant growth medium by the above criteria through physico-chemical analysis and plant growth trials.

Of major importance is the analysis of worm worked digested solids in this respect. Previous chapters have shown that digested separated solids can support earthworm growth, and so theoretically make feasible the linking of anaerobic digestion and earthworm culture as a waste treatment process. However the upgrading of digester residue by earthworms depends crucially on the value of worm worked material, which is highest as a plant growth medium. The chapter therefore includes work comparing digested and undigested worm worked material for plant growth.

The characteristics demanded of earthworm worked material as a plant growth medium are very different to those required by earthworms from separated solids. From the point of view of a plant the material can be divided into two convenient parts. First there is the undegraded fibre which forms the physical constituent of the material to allow roots to proliferate and support the plant. The most important characteristic the fibre must have is a high moisture holding capacity. Secondly there are the plant nutrients. The full complement of macro and micro nutrients must be present in the correct ratio and not in excess.

As a plant growth medium the worm worked material must be as inert as possible. If the fibre is capable of further decomposition it will shrink as mass is lost as CO_2 , possibly exposing the rootball. To break down the fibre, ions and especially nitrogen will become unavailable for plant uptake. The steady mineralization of organic nitrogen and phosphorus is desirable however.

Therefore from the study of digested and undigested solids before and after earthworm working, evidence that the fibre fraction of the material is stable, that plant nutrients are present, but not in excess, and that general values such as pH and ionic conductivity are within plant tolerance limits are all sought to theoretically test the suitability of the materials as a plant growth medium.

The literature cited in section 1.9.4 shows the paucity of plant growth data to support claims for earthworm worked material as a horticultural product, although earthworm catalysed decomposition processes reported in chapter one, such as an increased rate of mineralization, humification, nitrification, breakdown of odourous materials and the destruction of enteric bacteria all support the hypothesis that earthworm worked material has the potential to fulfill the role of plant growth medium.

Tomatoes, peppers and radishes are used experimentally as a basic horticultural crop to test this hypothesis.

10.2) Comparison Between Digested and Undigested Worm Worked Material as a Potential Horticultural Growing Medium.

10.2.1) Introduction.

The value of the residue produced from anaerobic digestion of agricultural wastes is an important factor in assessing the overall economic potential of this system as a waste treatment process. Several studies have indicated that anaerobic digestion is uneconomic at present when methane production and utilisation is costed as the main output from the system (Anon 1982, James and Campbell 1983).

However, if the residue from the digester could be upgraded above its crude fertiliser value when landspread the economics of the system could be greatly improved. One such way is the use of earthworms to produce a horticultural growing medium from separated digester residue, with a subsequent increase in value.

In previous sections it has been shown that *E.fetida* can grow and reproduce in digested cattle solids as well as it can in undigested material, and so process it into a form amenable to use as a plant growth medium. In this section it is proposed to study the differences between undigested and digested separated cattle solid based worm worked material which are examined in terms of their nutrient and physico-chemical characteristics to determine whether the fibre fraction of the material is stable, that major plant nutrients are present, but not in excess, and that general values such as pH and ionic conductivity are within plant tolerance limits.

10.2.2) Materials and Methods.

Fresh separated solids were collected from two sites described previously, Bore Place and NIRD. At each site the solids collected from slurry were derived from undigested and

digested slurry.

The fresh samples were frozen at -12°C , and at the earliest opportunity analyses as described in section 2.3.3. In addition the forage fibre analysis method of Van Soest (1971) was carried out to give a measure of the form and amounts of fibre in the solids. This is described in detail in the next section.

Approximately 20 litres by volume of both types of separated solid was placed in a storage bin with aeration holes and turned twice weekly to maintain the waste in an aerobic condition and allow mesophilic decomposition to take place.

The solids were bioassayed with earthworms to determine their acceptability. During each period of mixing five 10cm^3 subsamples were taken at random from the solids and placed in 10cm diameter containers. Five healthy adult *E.fetida* were placed on the surface of the solids in each container, and observed over a two hour period before being left overnight for another period of observation. If any earthworms died or remained on the surface, then it was deemed unacceptable. In practice, if earthworms did not show signs of immediately moving away from the negative stimulus of light into the solids, this indicated that the material was unacceptable.

Once the solids were found to be acceptable they were subjected to further analysis as before. The results are shown in tables 10.2.4.1 to 3. The acceptable solids were then placed in wooden earthworm breeding boxes, dimensions $45 \times 45 \times 15 \text{ cm}$. The boxes were stocked with a high earthworm population for rapid solids breakdown. After a period of two weeks the material had passed through the gut of the earthworm population and earthworms were removed by handsorting prior to further analysis of the resultant worm worked material.

10.2.3) Results.

Table 10.2.3.1 shows the nutrient status, and tables 10.2.3.2 and 3 shows the fibre composition of fresh and worm worked solids.

Table 10.2.3.1.

Plant Nutrient Analysis of Fresh Solids
and Worm Worked Material.

Material	pH	Ionic C.	P	K	Mg	NO ₃ ⁻	NH ₄ ⁺
		(uS/cm)	all ion concentrations in mg/l				
Fresh NIRD Undigested Solids	7.4	1024	97	864	92	13	282
Fresh NIRD Digested Solids	8.1	1070	180	1080	56	13	402
Worked NIRD Undigested Solids	7.7	832	112	1382	53	26	6
Worked NIRD Digested Solids	7.7	788	109	1353	43	24	6
Fresh Bore Place Undig- ested Solids	7.7	1517	202	1656	110	19	348
Fresh Bore Place Digested Solids	8.6	1851	208	1746	30	14	1080
Worked Bore Place Undig- ested Solids	7.8	1280	252	2045	110	28	6
Worked Bore Place Digested Solids	7.7	1448	252	2419	98	28	6

Table 10.2.3.2.

Van Soest Fibre Composition of Fresh Solids.

% Total Solids	NIRD Undigested	NIRD Digested	Bore Place Undigested	Bore Place Digested
N.D.F.	78	81	74	78
A.D.F.	49	59	48	55
Hemicellulose	29	22	26	23
Cellulose	32	30	28	32
Lignin	15	28	19	22
Ash	2	1	1	1
Total Kjeldahl Nitrogen	1.2	1.1	1.5	1.4

Table 10.2.3.3.

Van Soest Fibre Composition of Worm Worked Solids.

% Total Solids	NIRD Undigested	NIRD Digested	Bore Place Undigested	Bore Place Digested
N.D.F.	66	72	65	69
A.D.F.	54	60	52	54
Hemicellulose	11	12	13	15
Cellulose	20	19	18	20
Lignin	33	39	33	33
Ash	1	2	1	1
Total Kjeldahl Nitrogen	1.7	1.5	2.3	2.2

10.2.4) Discussion of the Differences Between Digested and Undigested Material as a Plant Growth Medium.

The majority of physico-chemical differences shown between these two materials have been noted in earlier sections and so the discussion here will focus on the changes in the fibrous material making up these solids.

The results obtained by the Van Soest method (tables 5.2.4.2 and 3) show a large percentage increase in lignin for NIRD solids from 15 to 28%, and an increase in Bore Place solids from 19 to 22%. Hemicellulose levels in NIRD solids dropped from 29 to 22% and in Bore Place solids from 26 to 23%. There was a 2% rise in cellulose levels for Bore Place solids compared to a 2% fall in NIRD solids.

Such results can be explained in terms of the original composition of the fibre content of the two materials, sawdust at NIRD and woodchips at Bore Place. The smaller particle size of sawdust gives a larger surface area for cellulolytic bacterial attack, and given the similar retention times for the digesters at both sites one would expect a greater degree of cellulose and hemi-cellulose breakdown for NIRD solids. If during digestion at Bore Place the relative loss of cellulose was very low compared to hemi-cellulose, volatile fatty acid, fat, carbohydrate and microbial decomposition, then this small actual loss could appear as an apparent percentage gain.

A visual inspection of Bore Place digested solids showed that some woodchips had passed through the digester intact. The type of wood is also an important consideration, as softwoods containing aromatic compounds could inhibit microbial growth. The woodchips used at Bore Place were purchased from a contractor who collected the woodchips from at least five local sawmills, and so a wide range of wood types could possibly be represented.

In both solids there was a similar loss of neutral detergent soluble material. These solubles are easily degradable during digestion, which over a 15 to 29 day retention time will be equally broken down in the digesters at both sites.

In terms of providing a stable physical base for a plant compost, the unreactivity of the fibrous fraction of the worm worked material is important. By this criteria the greater the percentage of lignin present, the less likely the material will be subjected to microbial decay, at least in the short term. Also important is the molecular alignment of the components of the fibre, the degree to which lignin shields cellulose and hemi-cellulose from decomposition. For example Robbins et al (1979) calculated that 44% of fermentable material in anaerobically digested straw based waste is shielded by lignin, and Summers and Bousfield (1980) found that 52% cellulose and 59% hemi-cellulose was not degraded in the anaerobic digestion of pig slurry with a 10 day retention time. That both digested and undigested solids from the two sites show large increases in lignin on worm working indicates its importance in stabilising fibre. On anaerobic digestion and subsequent worm working NIRD solids have a higher lignin level than undigested material, suggesting a combination of these two treatment processes produces a carbonaceous fibre in the resultant material less likely to experience short term microbial decay than worm working alone. This increase in lignin does not occur in Bore Place digested worm worked material, but in this situation the lignin may be in more intimate contact with cellulose and hemicellulose, and in this way reduce short term microbial decay.

In the long term all fibre in worm worked material will be subject to further microbial decay through the process of humification, but in the relatively short time period when the material would be used for plant growth this process will not be important.

The changes that occur to relevant plant nutrients on worm working can be seen in table 10.2.4.1. There is a general reduction in ionic conductivity on worm working, driven mainly by a loss of NH_4^+ ions through the process of volatilisation and nitrification,

the latter of which can be observed through the doubling of NO_3^- ion concentration in worm worked material and the relative increase in total kjeldahl nitrogen despite the large loss of NH_4^+ ions. Inorganic phosphorus levels remain relatively constant although one would expect mineralization of organic phosphorus on worm working (Satchell and Martin 1984).

For all treatments and especially Bore Place material there are high concentrations of potassium ions with a further relative increase on worm working as carbon is lost through microbial and earthworm respiration. Why potassium should show this relative increase when other ions do not is unclear. Perhaps phosphorus and magnesium have been incorporated into earthworm biomass to a greater degree and have therefore been removed from the system. Although potassium is a major plant nutrient, its presence in a plant growth medium in such high concentrations may cause problems.

10.3) Plant Germination Responses in Digested and Undigested Worm Worked Material.

10.3.1) Introduction.

The growth pattern of most plants go through three overlapping phases; germination, from seedling to young plant, and then to maturity, with each phase requiring different conditions for efficient growth. The ability of worm worked material to meet differing plant requirements at different stages of growth in terms of physical structure, water holding capacity and plant nutrients will allow a practical assessment of the material as an overall plant growth medium, and indicate any further treatment necessary to meet the demands for plant growth.

The seeds of three horticultural plant species, tomato, radish, and capiscum are used to measure the rate and percentage germination in undigested and digested worm worked material with Levingtons multi-purpose compost as a control. This is deemed a fair control material, because although seed composts recommended for germination have a low nutrient status, it is known that worm worked material has variable amounts of nutrients. As its name suggests, Levingtons multi-purpose compost is recommended for various roles, including that of seed compost, although it contains plant nutrients to enable it to be used in other functions.

10.3.2) Materials and Methods.

The following seed types were used;

Plant	Variety	Supplier
Tomato	Sonato (F1 hybrid)	Johnsons
Radish	Scarlet Globe	Johnsons
Capsicum	World Beater	Suttons

For each seed type three trays of dimensions 21 cm by 36 cm were threequarters filled with Levingtons multi-purpose compost and digested or undigested Bore Place worm worked material, obtained from laboratory cultures set up for eight weeks before the removal of earthworms by handsorting. The seeds were planted according to the following pattern;

Tomato; 3 rows of 7 seeds

Radish; 4 rows of 12 seeds

Capsicum; 3 rows of 7 seeds

The appropriate material was then added to the trays to bury the seeds to a depth of 1cm and gently pressed down, the trays were then watered thoroughly, covered with clear plastic film and placed in a source of natural light with the temperature controlled between 18 and 21°C.

The clear film was removed on the emergence of the first seedling, and from that period the trays were watered on demand. Emergence was said to have occurred when both cotyledons were visible. Physico-chemical characteristics of the material were analysed as in section 10.2.3.

10.3.3) Results.

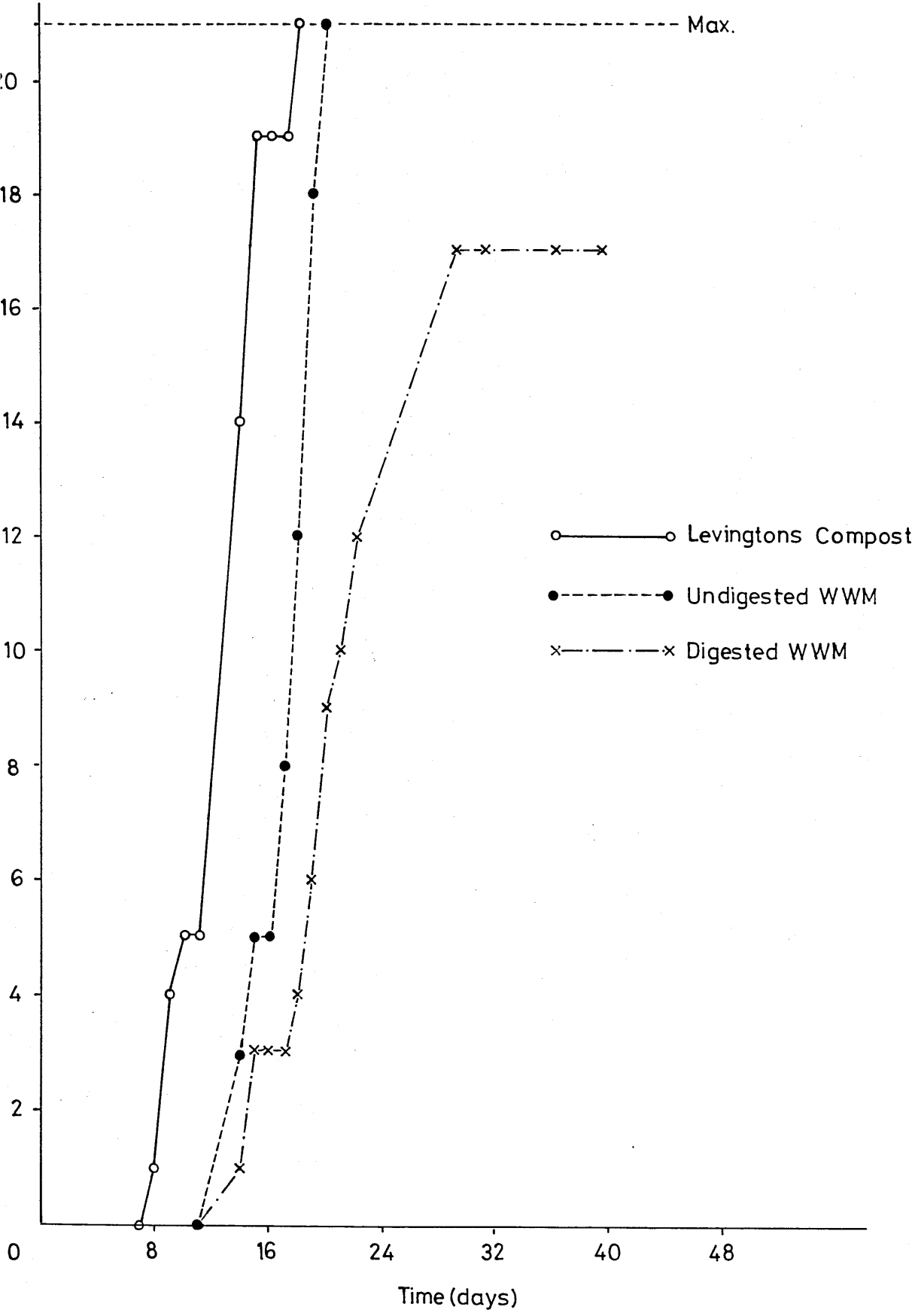
Table 10.3.3.1 shows the physico-chemical analyses of the two test materials and the control, and graphs 10.3.3.1, 2 and 3 show the rate of seedling emergence from the media for tomato, capsicum and radish respectively.

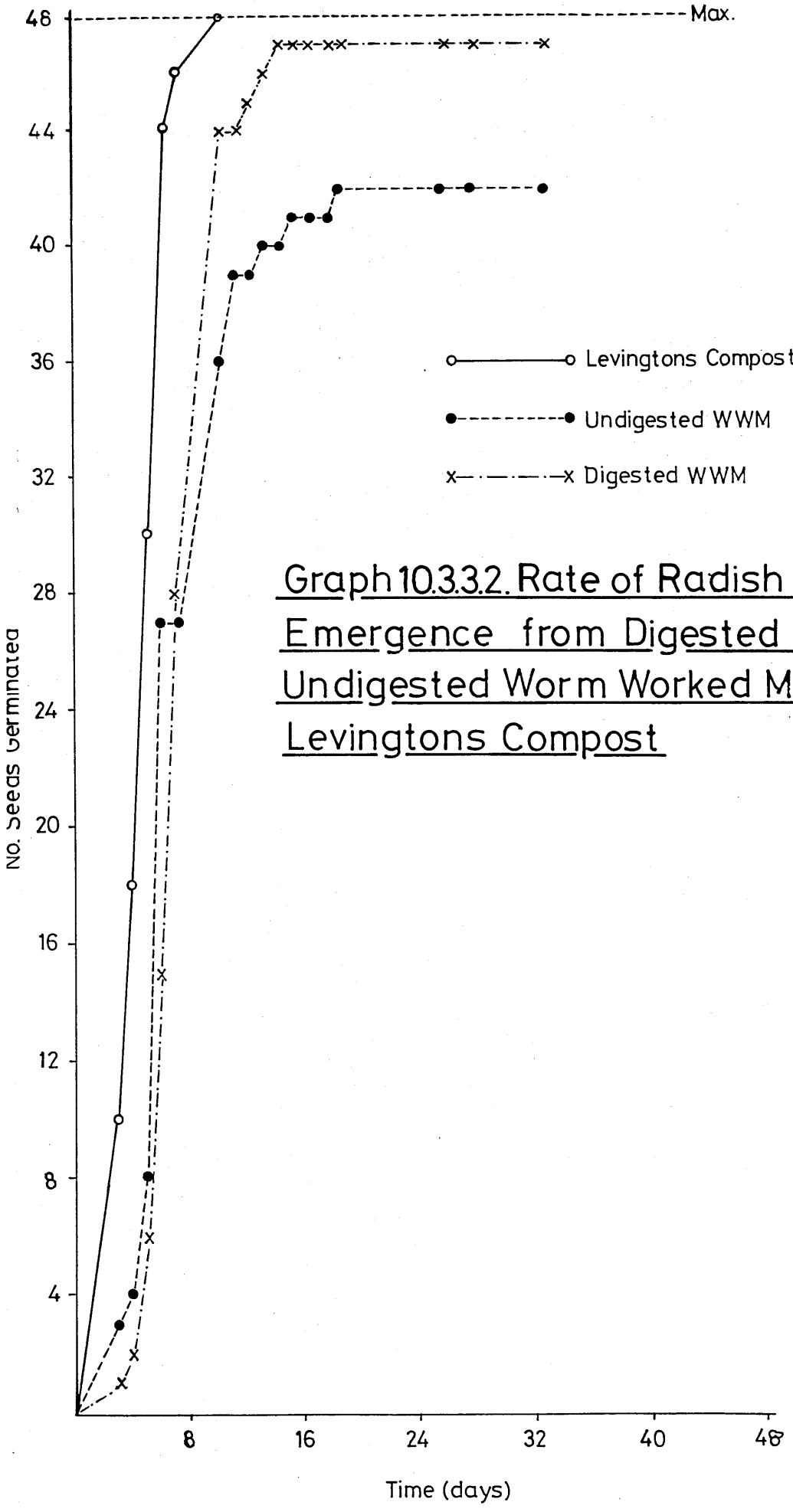
Table 10.3.3.1

Physico-chemical Analysis of Materials.

Variable	Compost control	Worm Worked Digested solids	Worm Worked Undigested solids
pH	5.8	8.0	7.3
Ionic C. (US/cm)	750	1593	1930
Inorganic P (mg/l)	184	144	320
K (mg/l)	720	2376	2959
Mg (mg/l)	100	138	178
NO ₃ ⁻ (mg/l)	<10	<10	<10
NH ₄ ⁺ (mg/l)	348	174	720

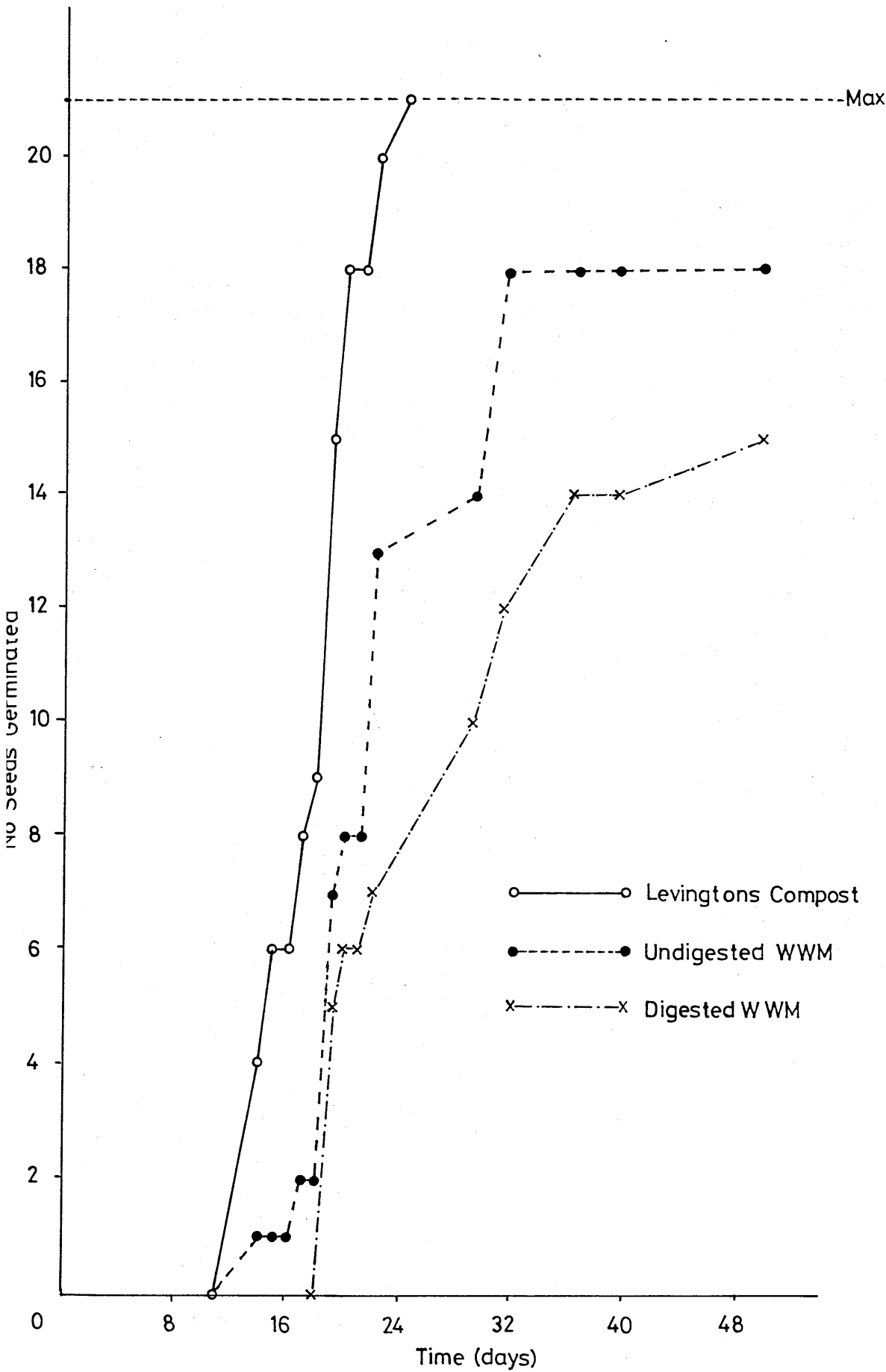
Graph 10.3.31. Rate of Tomato (F. Sonato) Seedling Emergence from Digested and Undigested Worm Worked Material and Levingtons Compost





Graph 10.3.3.2. Rate of Radish Seedling Emergence from Digested and Undigested Worm Worked Material and Levingtons Compost

Graph10.3.3.3. Rate of Capsicum Seedling Emergence
from Digested and Undigested Worm Worked
Material and Levingtons Compost



10.3.4) Discussion of Results.

From graphs 10.3.3.1, 2 and 3 it can be seen that for all three plant species Levingtons compost produces the fastest germination response, with 100% emergence in each case.

With tomato and capsicum, undigested worm worked material produced a faster germination rate than digested solids. Percentage emergence figures were 100% and 86% respectively for undigested solids and 81% and 71% respectively for digested solids. However for radish digested worm worked material produces a faster germination rate than undigested worm worked material. The percentage emergence values were 98% and 88% respectively.

Table 10.3.3.1 shows that overall both digested and undigested worm worked material differ from Levingtons compost by having higher levels of nutrients, especially potassium, which is over three times higher in worm worked material than the control. Magnesium levels are also higher, but not to the same degree. For phosphate and ammonium ions, undigested worm worked material contains higher concentrations than the control, but digested worm worked material has lower levels. The measured P values only record inorganic P salts, and, as the literature shows in section 1.2, up to half the P in animal wastes can be in the organic form which will be slowly released over time.

The overall level of ion concentration, as measured by the ionic conductivity, is approximately twice as high in digested worm worked solids and two and a half times as high in undigested worm worked solids when compared to the compost control. These high ion concentrations are most likely to be responsible for the lower germination rates in the worm worked solids. High ionic concentrations in the medium will impede the uptake of water into the seed which is vital for germination to occur.

However there must also be other factors affecting the germination rate in worm worked solids, because in two out of the three experiments undigested solids produced better results

than digested solids, but with a higher conductivity. It is possible that the higher pH of digested solids may have suppressed germination. A pH of 8 is beyond the upper limit recommended for most general crops (ADAS 1984). Radish seeds used in this experiment responded well to digested solids, with fewer losses than undigested solids, which indicates that radish seeds may be more pH tolerant than tomato or capsicum.

The results show beyond doubt that worm worked cattle solids cannot be used without some form of pretreatment to reduce the high pH and conductivity levels especially those recorded in the undigested worm worked solids. This could be achieved in one of two ways. Firstly, the material could be leached with water to remove some of the ions in solution which make up the high conductivity levels. The amount of water passed through the solids could be controlled to produce the required level of conductivity. In this situation the nutrients within the solids are seen as undesirable, and leaching is a way of removing them. By washing out cations such as NH_4^+ leaching may also reduce the pH of the material.

Another method is to dilute worm worked material with an inert or fairly inert medium such as peat, vermiculite, shredded wood bark etc. In this case the nutrients are not lost, but two materials with opposite characteristics are brought together to produce a composite form. In the case of peat its acidity would be advantageous in neutralising the more basic worm worked material, to reach a preferable pH of 6.5. This is tested in the next section.

Because seeds can easily be germinated in nutrient free media the extra treatment required to make worm worked material an acceptable product for germination may not be worthwhile. However, there is a place for a germinating medium that seedlings can be left in for some time into their life cycle before transfer, and such a material would obviously contain nutrients. It is for this type of product that worm worked material could be used, with suitable pre-treatment to optimise the nutrient levels therein. The

material would also have the advantage of having no inorganically based nutrients within it, and so being able to be labelled as an 'organic' growing medium.

10.4) Pretreatment of Worm Worked Material to Improve Characteristics for Plant Seed Germination.

10.4.1) Introduction.

Previously the germination of radish, capsicum and tomato seed was shown to be inhibited in digested and undigested worm worked material (WWM) when compared to a Levingtons compost control. The high ionic conductivity and pH of WWM was identified as a possible cause of inhibition, and two possible treatments were suggested, leaching with water and bulking with an inert material to favourably alter the physico-chemical characteristics of WWM for germination.

In this section plant growth trials are carried out in WWM in admixes with peat, an easily obtainable material already much used in horticulture.

10.4.2) Germination Trials of Worm Worked Material/Peat Admixes; Materials and Methods.

Earthworm worked Material produced from laboratory earthworm cultures grown in undigested solid from NIRD was collected and thoroughly mixed with commercially obtained Irish moss peat on a volume to volume basis to produce three admixes, 100%, 75%, and 50% WWM with peat. These were subjected to physico-chemical analysis as in section 10.2.2.

The admixes, along with two controls of Levingtons multi-purpose compost and 100% peat were placed in 21 x 36cm seed trays. 21 seeds of either tomato (Ailsa Craig) or radish (Scarlet Globe) were covered to a depth of 1cm in the growing medium. Four replicates of each treatment were set up. The trays were placed in a greenhouse and watered through a very fine rose to avoid disturbing the surface of the composts and

exposing seeds on the surface where they may have been liable to damping off or may have germinated more quickly. The trays were watered on demand, and examined every day for evidence for germination. This was said to have occurred when both cotyledons were observed to be clearly free of the germinating medium. After 19 days the experiment was terminated, and the seedlings carefully teased from the growing medium and washed to allow a final total dry weight measurement of plant shoot and root to be calculated for each treatment.

10.4.3) Results.

Table 10.4.3.2 shows total dry weight of plant and shoot for each admix. The total tray root and shoot dry weight in the different media was analysed statistically using Students t-test. The results are presented as a matrix in table 10.4.3.3. Weed seeds also germinated in some trays and the numbers in each treatment are included in table 10.4.3.2. The rates of seed germination are shown in graphs 10.4.3.1 and 2.

Table 10.4.3.1.

**Physico-Chemical Characteristics of Worm Worked Material
in Various Admixes with Peat.**

Variable	pH	T.S. (%)	V.S. (%)	Total N (mg/l)	Density (g/cm ³)	Ionic C. (μS/cm)
Levingtons	6.15	31.4	81.4	16.7	0.32	434
Peat	6.33	38.3	89.8	10.9	0.44	210
100% WWM	7.51	21.2	79.3	20.9	0.37	813
75% WWM	6.64	25.2	87.6	19.6	0.39	497
50% WWM	6.28	29.3	84.8	19.2	0.39	634

Table 10.4.3.2.

**Mean Tray Dry Weight for Radish and Tomato Seedlings Grown in
Several Media and Number of Germinating Weed Seeds.**

Medium	Seedling Dry Weight (g)		No. Weed Seeds	
	Radish	Tomato	Radish	Tomato
Levingtons	5.53	1.86	3	3
Peat	1.25	0.39	0	6
100% WWM	2.97	0.64	15	6
75% WWM	6.75	1.91	1	4
50% WWM	8.00	0.90	12	4

Table 10.4.3.3.

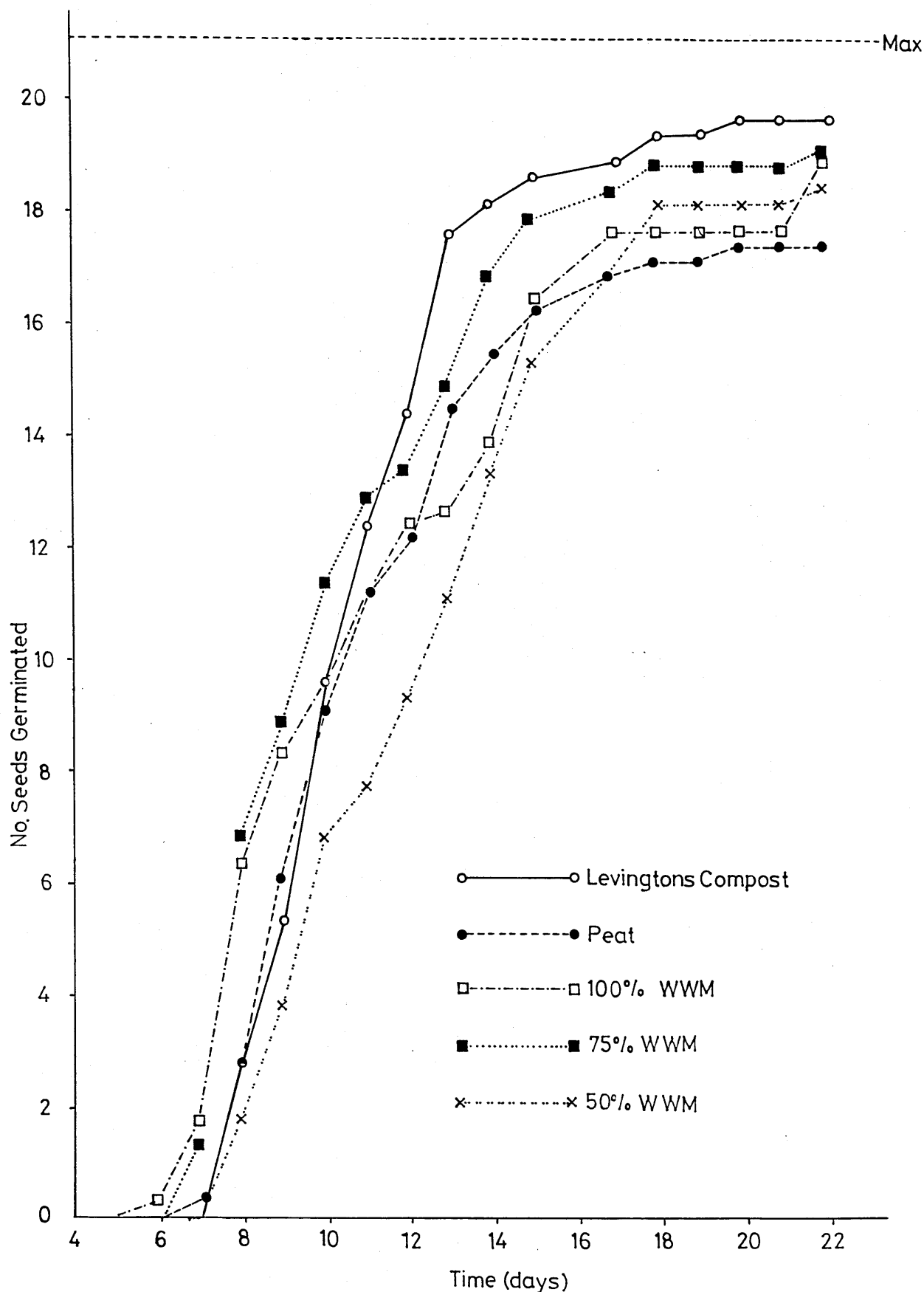
Students T-test Matrices of Total Tray Root and Shoot Weight
for Tomatoes and Radishes in Different Growing Media after 19 Days.

(Four replicates, taken at the 5% level of significance)

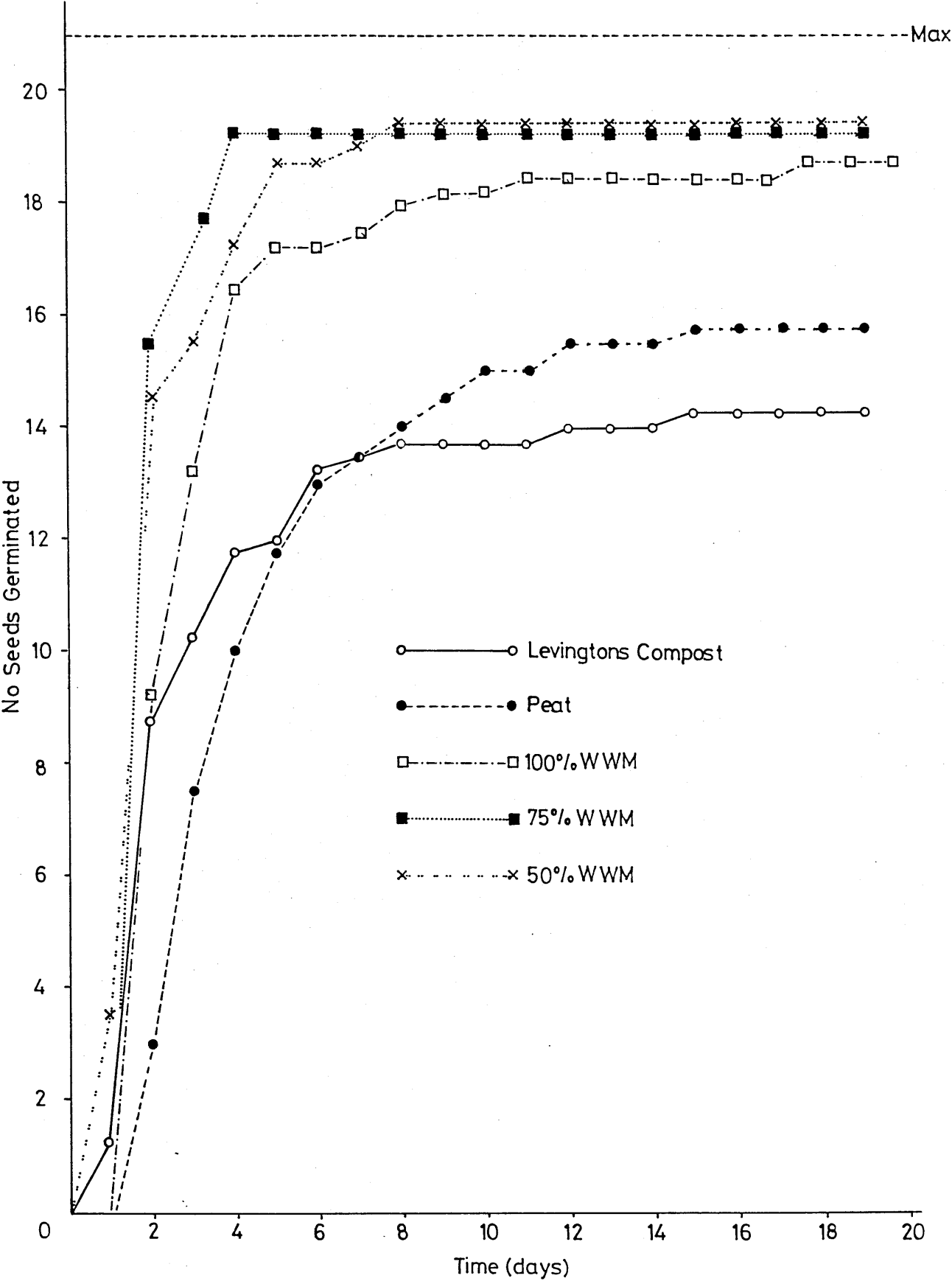
Tomato				
	Control	Peat	100% WWM	75% WWM
Peat	^			
100% WWM	^	-		
75% WWM	-	<	<	
50% WWM	^	<	-	^

Radish				
	Control	Peat	100% WWM	75% WWM
Peat	^			
100% WWM	^	<		
75% WWM	^	<	<	
50% WWM	-	<	<	<

Graph10.4.31. Rate of Tomato (Ailsa Craig) Seed Germination in Worm Worked Material in Different Admixes with Peat and Two Controls



Graph10.4.32. Rate of Radish Seed Germination
inWorm Worked Material in Different Admixes
with Peat and Two Controls



10.4.4) Discussion of Results.

Taking the results as a whole there is no doubt that the pretreatment of WWM through dilution with peat has affected the germination results, with the effects more obvious for radish than for tomato.

The graph of tomato seed germination shows little difference between the five media. The standard error bars have been omitted for clarity as they generally all overlap. Although Levingtons compost produces the final highest number of germinating seeds, no clear pattern of germination differences between the three WWM admixes can be observed. The dry weight of tomato seedlings per tray shows 75% WWM produced a significantly higher weight than any other treatment apart from Levingtons compost. These results highlight the apparent conflict between the roles of a seed compost, which must provide optimum conditions for seedling growth until they are strong enough for picking out, and indicate that for tomato the negative and positive factors of peat and WWM in terms of seed germination and seedling growth cancel each other out to produce a medium giving equally good results when compared with Levingtons multi-purpose compost. The important physico-chemical factors are the low ionic conductivity and the low total nitrogen content of peat and thus it's low nutrient content, compared with the high pH, total nitrogen and ionic conductivity of WWM.

There is a much shorter lag phase in the germination of radish seeds, appearing after the first day compared to five days for tomato, and a much greater difference between the results of the different media. Both peat and Levingtons multi-purpose compost appear to inhibit radish seed germination when compared to the various WWM admixes. The mean radish weights at the end of the experiment also show significant differences between treatments. Radish plants germinate and grow faster than tomato plants, giving a plant biomass about five times higher than tomatoes at the end of the experiment. This indicates

that in its twin role, the balance is tipped towards the subsequent growth of the seedling for radish. This can be seen from the data where the mean total plant weight in Levingtons is significantly higher for all treatments except 50% WWM, even though fewer seeds finally germinated. On the other hand every treatment produced significantly greater total plant biomass than peat, where the lack of nutrients was especially felt.

Although 100% peat produced poor results, the higher its percentage inclusion in WWM mixes the greater the final plant biomass. Thus 75% WWM produces a significantly higher mean weight than 100% WWM, and 50% WWM produces significantly better results than either. However, studying the physico-chemical values of 50% and 75% WWM there appears to be little difference between the two, indicating the precise environmental optima required for germination and initial growth of radish.

That tomato and radish plants produced the final maximum plant weights in 75% and 50% WWM respectively shows they have different requirements from the environment, and emphasises the problem in using a material such as WWM. The results of mixing WWM with peat show that it is possible to alter the characteristics of the material fairly easily to obtain respectable results. Given the innate variability of an animal derived medium such as WWM (the results in section 10.2 cannot be directly compared for example as it was material from a different site collected at a different time and therefore having very different characteristics) the results indicate that different mixes would be required for different plant species and for WWM derived from different sources. This innate variability suggests that each batch of WWM would require analysis of key physico-chemical factors and then be treated in a variety of ways, possibly through additives, to reproduce consistent conditions for a particular purpose. Whether this is within the scope of a commercial operation is beyond the bounds of this thesis.

Another minor problem is the greater incidence of weed seeds germinating in the seed trays of WWM (although Levingtons and peat are also affected). Although no more than

a nuisance, a processing system would also have to consider ways of reducing foreign seed viability within a waste derived material.

10.5) Plant Growth Trials in Digested and Undigested Worm Worked Material

10.5.1) Introduction.

In previous sections untreated worm worked solids were found to inhibit seed germination, but when diluted with a bulking agent better results were obtained. It was concluded that the high nutrient contents in the worm worked material were involved in the inhibition process. Nutrient levels may therefore be more suitable for plant growth after germination has occurred. This hypothesis is tested by growing tomatoes from seedlings to fruiting in digested and undigested worm worked solids, again using Levingtons multi-purpose compost as a control. The physical size of the plants and the yield of tomatoes per plant were measured to judge the ability of worm worked material to support the growth of a horticultural crop.

10.5.2) Materials and Methods.

F1 (sonato) tomato seeds were germinated in trays and transplanted into 10cm and later 15cm plastic pots before being transferred to polyethylene growing tunnels. Plants were watered on demand, and when the first truss had set a proprietary liquid fertiliser (NPK 4-4.5-8) was fed at every other watering. The tops of the plants were picked out after the fifth truss had set. 16 replicate plants were used for every experimental treatment. Tomatoes were picked when ripe and weighed individually.

10.5.3) Results.

Table 10.5.3.1 shows the height reached by tomatoes after 95 and 105 days, and the number of leaves and trusses formed. Table 10.5.3.2 shows the final yield of tomatoes from the plants and the percentage that were disease damaged.

Table 10.5.3.1.

Physical Characteristics of the Plants after 109 days.

Variable	control	Worm worked Digested solids	Worm worked Undigested solids
Mean height (cm)	125	41	58
Standard Deviation	20	14	14
No. leaves	20	14	14
No. trusses	4	1	1

Table 10.5.3.2.

Final Fruit Yields of Plants.

Variable	Control	Worm worked Digested solids	Worm worked Undigested solids
Mean no. fruits per plant (\pm SE)	40 (1.1)	35 (1.5)	30 (1.7)
Mean total fresh weight per plant (g) (\pm SE)	2356.4 (87.9)	1097.5 (97.8)	1275.8 (69.6)
Mean Individual fresh weight per plant (g)	58.9	31.4	42.5
% disease damaged (by total weight) (\pm SE)	12.5 (1.9)	39.5 (3.6)	21.7 (1.3)

10.5.4) Discussion of Results.

A comparison of tables 10.5.3.2, 3 and 4 shows that tomatoes grown in Levingtons multi-purpose compost produced the highest yield of fruits per plant with the highest overall weight. Plants grown in undigested worm worked material produced a higher number of fruits compared to digested worm worked material, but with a lower total weight.

Using the analysis of variance on the mean total yields of the three treatments (2356.4g, 1275.8g and 1097.5g respectively) it was found they were significantly different at the 5% level of significance.

Plants grown in undigested solids also had an unacceptably high number of fruits suffering from disease damage, either blossom end rot (BER) or a scaliness of the skin (39.5% by weight compared with 21.7% in digested solids and 12.5% in the compost control). These results were also significantly different at the 5% level of significance using an analysis of variance.

The results therefore show that both digested and undigested worm worked material produce significantly lower yields than a commercially available compost control, and with a higher incidence of disease which would make the fruit unsaleable. However none of the plants in any of the treatments showed any micronutrient deficiencies nor viral or fungal disease symptoms

The causes of BER in tomatoes are not completely understood, but it is generally accepted to be caused by a local deficiency of calcium in the fruit of the tomatoes (Shear 1975, Simon 1978). The plants are therefore likely to be suffering from reduced calcium ion transport from the growing medium associated with generally reduced water uptake, as observed by Stuckey and Temple (1911). Work in the previous section shows that digested

and undigested worm worked material have a high ionic conductivity compared to the compost control, mainly through an accumulation of potassium ions within the solids. High ionic conductivity levels within the growing medium will inhibit the rate water uptake by plant roots, and so reduce the transport of calcium to the set fruit. Robbins (1937) found that increasing the osmotic concentration of the nutrient solution increased BER in tomatoes. Geraldson (1967 and 1973) showed that BER incidence in tomatoes was correlated with a low calcium to total soluble salt solution ratio, with the effectiveness of other ions in causing this effect in the order $\text{NH}_4^+ > \text{K} > \text{Mg} > \text{Na}$. NH_4^+ ions will be released from organic nitrogen on mineralization, and potassium levels have been shown to be high.

High levels of salt in the growing medium can also inhibit root growth throughout the medium and so limit water uptake and growth. A visual inspection of the root balls of the plants at the end of the experiment showed a reduced root system for plants in digested solids and even more so for plants in undigested solids

Undigested worm worked solids had a less well defined crumb like structure when compared to digested worm worked solids. During the course of the experiment the undigested material also shrunk and so lost volume. This suggests that the undigested solids had not reached a stable stage of decomposition. This was not seen for digested solids and indicates that a combination of anaerobic digestion and worm working had stabilised the material more fully. The combined effects described above would tend to reduce the moisture holding capacity of undigested worm worked material and so enhance the rate of blossom end rot.

Both the high pH and ionic conductivity evident in digested and undigested worm worked material could account for the reduced yields for tomatoes grown in these media. It is interesting to note that plants in undigested worm worked material produced a higher number of fruits per plant, but with each having a much lower mean weight compared

to digested solids. Digested worm worked solids have a higher pH which may have been the main factor in the inhibition of tomato yields. Possibly the lower pH in undigested solids allowed a greater number of fruit to be set, but the onset of BER caused by the high conductivity levels and the subsequent lower mean weight per fruit produced a lower total yield. With a high percentage of tomatoes being made up with water anything affecting the uptake of water into the fruit is likely to have an influence on yields.

In conclusion, with yields half that of the control plants and a twofold increase in disease symptoms it is obvious that neither digested nor undigested worm worked material could be used as a horticultural growing medium in their untreated form. This is not surprising as all commercially available growing composts are especially prepared and formulated to provide a nutritionally balanced growing medium. It is unreasonable to expect worm worked material to produce equally good results without a similar process of pretreatment.

This experiment shows that animal derived worm worked solids can support plant growth with further pretreatment to balance the macronutrient levels and pH. From the evidence of this experiment micro-nutrient levels are satisfactory and no action need be taken against pathogens. Advantage could be taken of the organically bound nutrients in worm worked solids, especially nitrogen and phosphate. Being released slowly over the course of the growing season, they could be of important advantage to container grown plants where the limited nutrient holding capacity of the compost is always problematical.

Chapter Eleven

General Discussions and Conclusions

11.1) Hypothesis Development During the Project.

The research for this project began by addressing a broad practical question; can earthworms be grown in available forms of agricultural organic matter, and can the factors affecting their efficiency of biomass gain be defined and manipulated. Interest centred on the ability of digested cow manure to support earthworm growth, with the possibility of combining anaerobic digestion and earthworm culture systems, and therefore making anaerobic digestion a viable process. There is only one reference to the use of anaerobically digested sewage sludge in the literature, which found the material to be toxic to earthworms (Mitchell *et al* 1980). However, there is evidence that the economics of anaerobic digestion could be improved if the value of the digester residue can be increased, through processes such as earthworm culture.

The facilities at Bore Place potentially allowed this question to be examined in the context of a farm where such forms of organic matter are already generated and where any application of earthworm culture techniques is likely to occur.

Field scale earthworm growth trials were therefore initiated to test the ability of mechanically separated undigested and digested cattle solids to support earthworm growth at Bore Place. Initial results did not support the theory that earthworms could be used to upgrade the material, as expected from the literature, but this initial experiment only represented one set of conditions under which earthworms can be cultured. Further trials were initiated to investigate if successful earthworm culture could occur under other conditions at field scale, but with negative results.

The next phase of work was therefore carried out in laboratory experiments, to allow scale up factors to be taken into account and allow a greater degree of control on the conditions prevailing in the waste medium. Here direct evidence was obtained that separated cattle solids from different sites produced significantly different earthworm

biomass gains independent of external factors, indicating specific variable characteristics within the waste that affect earthworms.

This changed the direction of the thesis away from a broad question of how to culture earthworms in cattle solids to allow a successful farm based system to operate, to the question of what intrinsic factors within cattle solids control the earthworm growth response. In theory such an approach would allow agricultural wastes to be physico-chemically characterised and appropriately treated to allow earthworm culture at the field scale to operate successfully and thus indirectly answer the initial question.

An associated question is how the actions of earthworms affect the properties and characteristics of waste materials, with the attendant practical benefits this can bring to the use of worm worked material as a horticultural product. This becomes more important when studies show that the ability of earthworms to convert inaccessible microbial protein to a harvestable earthworm protein is of no economic value.

Earthworms cultured in organic matter become part of a complex decomposition system undergoing a series of interactions between all components, living and dead, organic and inorganic. The questions are; can any of the interactions between earthworms and organic matter be measured and quantified, can the information gained be used to interpret variations in earthworm growth, for example between digested and undigested solids, and can worm worked organic matter be used as a plant growth medium.

11.2) Earthworm Growth in Bore Place Material.

The products of earthworm culture, earthworm protein and a worm worked material must have a sum value greater than that of the original material for the system to be economic. Earthworm trials at Bore Place were set up before economic studies discounted earthworm protein as a viable end product. There was also an assumption that although agricultural wastes derived from various animals had different properties, the cattle solids at Bore Place could be considered as representative of separated cattle solids in general, and results extrapolated to separated cattle solids derived from other sites.

It became apparent from the first set of field scale earthworm growth experiments that the results obtained were not consistent with earthworm culture results from the literature. However, as these were the first experiments carried out, many factors could be involved, and so other field scale trials were initiated with variations in overall conditions and treatments. Factors such as the depth to which the bed was loaded, the temperature of the bed, the stocking density and the way in which the materials was composted prior to earthworm inoculation were all found to affect earthworm growth. However, the results did not help to answer the general questions regarding improvements in earthworm culture efficiency. Earthworm growth appeared to be influenced by intrinsic properties of the substrate affected by the type of cattle providing the slurry, their diet, the type of bedding, slurry storage facilities etc. and changing the culture method of the earthworms did not influence these.

One way to examine these varied factors is via the physico-chemical characteristics they produce in the separated cattle solids. Understanding the physico-chemical processes in organic materials occupied by earthworms is the key to manipulating the efficiency of earthworm culture. Earthworm culture management aims to alter the physico-chemical characteristics of the waste in a manner which is of benefit to earthworms.

Physico-chemical factors affecting earthworms growing in Bore Place solids can be divided into intrinsic and extrinsic factors. The field work varied extrinsic factors such as moisture content and temperature. Both these factors exert a direct effect, e.g. high temperatures cause mortalities and a high solids moisture content creates anaerobic conditions. The literature shows 20-25°C as an optimum with 35°C as an upper survivable temperature limit for earthworms, but this work shows that a heterogeneous temperature regime can develop in a large body of waste. Therefore temperature has an indirect effect on the spatial distribution of earthworms within solids. High temperatures cause earthworms to move to the edge of a bed where lower ambient temperatures allow more tolerable conditions to develop.

Higher moisture contents cause a build up of water in the base of beds if drainage is impeded and so allow anaerobic conditions to develop. However, the amount of water a solid can hold before conditions likely to cause earthworm mortalities develop depends very much on the physical structure and pore size distribution of the material. Single source bulking agents such as woodchips or straw caused mortalities at 85% moisture content, but this value produced maximal growth in separated solids. Earthworms can survive in almost saturated conditions if they have the opportunity to move in and out of the affected areas, and are not stressed by other non-optimal conditions. Quoting a particular moisture content in relation to earthworm performance is almost meaningless unless the material it relates to is also mentioned.

Changes in management regimes of field scale earthworm beds allowed factors such as moisture content, temperature, stocking ratios etc. to be altered, but with little increase in earthworm biomass above the initial stocking density. This indicates that physico-chemical factors that are intrinsic to the nature of the solids were probably inhibiting earthworm growth. Laboratory experiments in Bore Place material showed better results than those obtained in the field, indicating that a detrimental scale-up factor also operated. Significant increases in biomass of earthworms occurred in separated solids

from other sites. This indicates some inhibitory effect operating on earthworms within Bore Place material. Like many biological systems the growth of earthworms in organic wastes is likely to be controlled by a series of limiting factors and these are discussed in the next section.

11.3) Limiting Factors Controlling Earthworm Growth in Organic Matter.

When discussing factors affecting earthworms in organic wastes it is useful to subdivide these into four groups. Nutritional and environmental factors can be further subdivided into organic and inorganic factors, and all these groups interact.

Early attempts to alter the physico-chemical characteristics in the animal wastes in order to test their effects on earthworms caused too many factors to change, making the interpretation of results difficult. There are two possible ways of overcoming this problem; to create artificial media from simple materials, the other to modify existing substrates in a controlled manner. The work on cellulose addition to cattle solids and on artificial media emphasised the complexity of organic materials and showed that crude changes such as the addition of cellulose produces limited results. Treatments such as anaerobic digestion and different aging and composting techniques were used to produce a range of different physico-chemical conditions within the material. The difficulty with this method is separating out the effects of the different individual factors but it allowed the separate effects of limiting nutritional and environmental factors to be identified from the earthworm growth pattern produced. Four features of growth pattern are important; initial growth rate, mortality, final total biomass and subsequent rate of loss. Limiting nutritional factors did not affect initial earthworm growth but reduced the overall biomass produced, and increased the rate of loss of weight. Limiting environmental factors, if severe enough, could cause earthworm mortalities or reduce the initial earthworm growth rate and possibly the final biomass value.

Ionic conductivity, a measure of overall ion concentration had a major effect as an environmental factor. The ability of nitrogen to change its form within organic wastes from large organic molecules (e.g. proteins) to an inorganic ion (NH_4^+) to a small molecule (NH_3), which can then change its state to gas, makes it an important contributor to ionic conductivity. The presence of ammonium ions in solids substantially add to the ionic

conductivity of separated solids, especially in fresh material. Ammonia losses over time through volatilisation allow the ionic conductivity to fall to a value where earthworms can survive. High ammonia levels may also have a specific toxic effect above that of adding to the ionic conductivity value, but this was not shown conclusively by the research.

Phosphorus can also change from an organic to inorganic form and thus affect ionic conductivity. Other ions which remain in the inorganic form cannot play an active role in changing the environment, but may be toxic to earthworms if any individual ion is present in excess. Although inorganic ions are a nutritional requirement of earthworms, none of the research carried out here indicated earthworm inhibition caused by lack of nutritional inorganic ions.

The general nutritional requirements of earthworms from organic materials can be crudely summarised by the C/N ratio. Discussion of nutritional factors concentrates on carbon because the nature of these organic materials is such that nitrogen is rarely limiting. Microbially driven mineralization and immobilization processes can also convert N into the most appropriate form. When the C/N ratio is above the 35/1 value it effectively makes most carbon unavailable for nutrition as micro-organisms are limited by nitrogen. The results of such a situation are seen in the FYM field experiment at the Open University when the earthworm population fell to almost zero in FYM with a high straw and low faeces and urine composition. This work has shown the importance of taking into account both available and non-available carbon. The optimum value of 23/1 in earthworm culture media was found to be lower than the 25 to 35/1 optimum quoted for composting, taking into account the presence of non-available carbon. However, nearly all carbon in organic wastes can become available given enough time. In the solids studied in this project a high proportion of carbon originates from the bedding material of the cattle and is possibly the single most important constituent of the material, affecting its physical characteristics and also providing a major nutritional input. Given its importance

experiments carried out using separated liquids and bulking agents are of relevance. The low availability of carbon associated with lignin in woodchips from Bore Place reduced the availability of nutritional carbon, and the relatively large particle size of the woodchips may have reduced subsequent microbial attack on recalcitrant carbon. This concept is supported by comparison with solids from NIRD which used sawdust with a larger surface area to volume ratio as a bedding material and produced better earthworm growth results. Ground woodchips produced a higher final biomass when used as a bulking agent with separated liquid compared to whole woodchips. Separated solids from slurry incorporating straw at Oaklands Agricultural College, having less lignin, produced better earthworm growth than solids from Bore Place and NIRD.

It must be emphasised that the physico-chemical factors discussed do not act in isolation but form part of an active whole. Indeed the work on solids manipulation and artificial media showed that when the earthworm environment was simplified in some way, either through adding a single source nutrient, or through trying to create an artificial medium, earthworm growth inhibition or even mortalities occurred. The reasons for this are discussed in the relevant sections, but generally a more complex medium is more stable, has more ecological micro-niches to support a varied microbial population and has feedback systems which counter large variations in physico-chemical factors that can otherwise cause inhibition or mortalities. The results from the literature show better earthworm growth in aerated, activated sludge than animal manures, and given the varied constituents and treatment processes that make up sewage sludge there is no doubt it makes up a more complex earthworm growth medium than animal manures.

11.4) Effects of Earthworms on Organic Materials.

Decomposition processes are driven by the activities of the faunal decomposer community, which will include bacteria, actinomycetes, fungi, protozoa, nematodes etc. When earthworms such as *E.fetida* are introduced into such a waste system, two questions arise; how much do they affect the rate of decomposition, and are there any changes in decomposing waste which only occur in the presence of earthworms.

Theory suggests that earthworms affect the organic material they inhabit by a variety of processes; mixing the material, aerating it, bringing fresh waste into contact with micro-organisms, comminuting ingested material and thus increasing the surface area for further microbial decomposition and also having gut enzymes to break down some of the components of the waste, with possibly a symbiotic gut micro-flora carrying out a similar process. However, work in chapter nine showed earthworms in separated solids under laboratory conditions produced no increase in the rate of change of selected physico-chemical characteristics. Although it is problematical to interpret results gained from pot experiments in the laboratory to larger field scale situations, these results do not support the idea of a major role for *E.fetida* in changing agricultural wastes through decomposition processes. When earthworms are present in such a system they quickly become integrated into the microbial processes occurring within the waste. If the microbial decomposition processes are occurring efficiently then one could argue that earthworms in themselves do little to enhance the process. The role of the earthworm in producing a horticultural medium can therefore be described as a basically mechanical task of mixing and aerating the solids and physically breaking down large particles.

Although work in chapter nine showed no increase in the rate of fibre decomposition in laboratory conditions, under field conditions a particle size reduction would expose fibre to further breakdown and thus produce greater stability in the final material. Because such a particle size reduction influences the feel and texture of the solids, this also has

considerable importance in marketing such a material. Organic wastes treated through composting alone do not undergo so much particle size reduction and they are therefore limited in use.

The work in chapter ten shows that worm worked material, if suitably treated, provides an adequate medium for plant growth, and being derived from a waste source may have price advantages over peat based composts. The main disadvantage in worm worked materials is the large nutrient variability. However, this has nothing to do with the actions of earthworms but is influenced by variability in the source material.

11.5) Earthworms and Anaerobic Digestion.

Earthworm culture is clearly compatible with the process of anaerobic digestion. Although anaerobic digestion affects the physico-chemical characteristics of the waste, it does not make such a material toxic to earthworms such as *E.fetida* except when presented fresh, a toxicity effect also seen in undigested solids. Experimental results show that digested solids produce varied responses in earthworms depending on such factors as the retention time in the anaerobic digester, the form of solids pre-treatment and the type of cattle bedding material used but it was not possible to indicate an optimal process.

Worm-worked material derived from digested solids can be used as a horticultural growing medium and the anaerobic digestion process prior to worm working may have the advantage of increasing the breakdown of celluloses and hemicelluloses which when combined with earthworm culture provides a more stable horticultural medium, less prone to further decompositional processes than similar materials produced from undigested waste.

11.6) Future Research Possibilities.

The central question of the thesis is; can any of the interactions between earthworms and organic matter be measured and quantified, and the information gained used to interpret variations in earthworm growth patterns and worm-worked material characteristics. Preceding sections have discussed the results of using varying techniques such as waste pre-treatment, manipulating particular characteristics of the waste, and trying to simplify and therefore manipulate more easily the earthworm environment through the use of artificial media. The work has identified several factors which deserve further attention, such as the ionic conductivity of the material, especially as affected by the ammonium ion, and the nutritional quality of the material as measured by the earthworm available C/N ratio. The question becomes one of which technique can best be used to study these factors.

Waste pre-treatment produced crude changes of too many factors to be useful for this sort of study and the use of artificial media have been shown to be untenable. Therefore, manipulation of individual factors within waste materials appears to provide the most fruitful approach. However, changing one factor in a complex ecosystem can upset the equilibrium and thus alter other characteristics. Initial experiments are required to investigate the interactions occurring on adding, for example, ammonium or other ions, and organic nitrogen or carbon compounds.

Two possible approaches are as follows; by dividing separated solids into a liquid and solid fraction two crude but possibly effective manipulations can be achieved. The liquid fraction containing dissolved ions and nutrients can be removed by repeated leaching with distilled water allowing subsequent replacement with ions at varying concentrations. Conversely the larger fibre particles of waste can be replaced by material of known composition and size, and liquid from the mechanical separation of the same slurry can be used to provide the soluble nutrients as this has the same composition as liquids within

separated solids. These two methods represent a compromise between using artificial media and directly adding material to wastes.

The information derived from such laboratory pot experiments could also be improved if techniques can be developed to allow measurements of micro-sites within the solids rather than measuring material that is bulked together to sample. Such techniques would allow study of material passing through the earthworm gut and earthworm faeces and could possibly be used to collect and analyse favoured earthworm feeding sites within the material. Identifying processes occurring at micro-sites may provide the key to understanding earthworm/organic waste interactions.

The importance of earthworms in the decompositional processes that occur in organic wastes as studies in this thesis needs to be seen in terms of the wider debate of the role of soil macro-fauna in processes such as litter decomposition and incorporation into the soil, humification, nutrient cycling etc. Given that such processes are ultimately carried out by micro-organisms, the question becomes one of how macro-fauna such as earthworms influence the micro-fauna in their activities, and whether the influence is significant in terms of the net overall effect on the decomposing system. Ultimately such questions are only going to be answered through multi-disciplinary studies into the relevant areas, of which earthworm culture in organic wastes is only a part.

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